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THEILERIA PARVA, THE PARASITE OF EAST COAST FEVER IN CATTLE.

OBSERVATIONS ON STAINED PREPARATIONS.

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(*From the Quick Laboratory, Cambridge.*)

(Plate XII and 2 Charts.)

IN an earlier paper (Nuttall, Fantham and Porter, 1909, *Parasitology*, vol II. pp. 325—340), we recorded our observations on living *Theileria parva* as seen in the peripheral blood of two cows which succumbed to East Coast Fever[1]. We now propose to describe our studies upon the parasite in stained preparations made from the animals' blood during the course of the disease and from their organs shortly after death. We shall confine our attention to the types of parasites encountered within red blood corpuscles or to corresponding types which may be occasionally encountered free in the plasma. The subject of "Koch's blue bodies" will receive attention at a later date.

Methods used in making preparations.

Blood films were prepared daily from the cows, beginning a few days after the pathogenic ticks (*Rhipicephalus evertsi*) had been placed upon the animals.

The blood was obtained in the usual way by puncturing an ear-vein; it was usually spread thinly on slides, rapidly air-dried, fixed in absolute alcohol and stained by Giemsa. We preferred this method of fixation to wet fixation methods because the latter yielded inferior

[1] For further details regarding the two cows above-mentioned, the reader is referred to *Parasitology*, vol. II. pp. 208—210 and p. 328.

results. The minute intracorpuscular parasites in wet-fixed films are only seen indistinctly owing to the intervening cloudy layer of stained haemoglobin in the corpuscles, which do not assume the flattened shape observed in ordinary films. Wet fixation was tried in various ways, by osmic acid vapour, by sublimate-alcohol, by Flemming's solution followed by staining with Delafield's haematoxylin, thionin, or safranin-methylene blue, etc., but the results were uniformly unsatisfactory.

The morphology of the parasites as observed in stained preparations.

The parasites vary much in size and shape, as will be readily seen by a glance at Plate XII. Parasites occurring singly in corpuscles may be of all shapes and sizes. When two parasites are contained in a corpuscle they may possess about the same shape and size (figs. 25, 26), but as a rule they differ considerably in both respects (figs. 21—24). This variation in size and form is very noticeable in corpuscles which contain larger numbers of parasites (figs. 31—45). Ovoid and bacilliform parasites (figs. 34, 36), or ovoid and comma-shaped parasites (figs. 32, 33), or, again, a cross-form, a bacilliform and a rounded parasite (fig. 48), etc., may be contained in one and the same corpuscle.

We append some measurements made on typical specimens of the parasites in well-stained preparations:

Ovoid	parasites	1 to 1·5 × 0·55 to 0·8 μ.
Rounded	„	1·5 to 1·7 × 1·1 to 1·4 μ.
Comma-like	„	2 × 0·7 μ.
Bacilliform	„	0·7 to 2·9 × 0·3 to 0·4 μ.

Although the shape of the parasites is very variable the commonest form is usually *ovoid or oval* (Pl. XII, figs. 1, 2). In typical parasites belonging to this category the chromatin mass occurs at one pole alongside of a clear area in the blue-staining protoplasm which appears condensed peripherally. This clear area may represent either a vacuole or tenuous protoplasm. *Rounded or ovoid parasites,* often of larger size than the foregoing (figs. 9, 24, 36), are not infrequently encountered with a clear central area and a crescentic or horse-shoe-shaped mass of chromatin situated at one pole, its curvature corresponding to that of the external contour of the parasite. Some of the horse-shoe-shaped masses of chromatin (figs. 10, 34, 35, 42, 48) present a bent dumb-bell-like appearance through concentration of the chromatin distally, the appearance suggesting that they are undergoing division. In other cases

(fig. 21) two chromatin masses appear to be separating, the interspace staining faintly pink. *Comma-shaped or club-shaped parasites* (figs. 5, 6) are very common. In these the chromatin mass is terminal and the protoplasm usually stains uniformly blue. *Straight or slightly curved bacilliform parasites* (figs. 13—16, 26, 27) are also seen, which closely approximate to the foregoing. In the earlier stages of the disease (figs. 5, 6, 7) such parasites may show relatively large chromatin masses and in some cases (figs. 8, 25) have a drum-stick appearance. At times the chromatin mass shows a slight constriction (fig. 28), suggesting commencing nuclear division. When two bacilliform parasites chance to lie cross-wise (figs. 29, 40, 41, 43), as occasionally happens, they may present the appearance of true cross-forms. Bacilliform parasites increase in numbers during the course of the disease. *Pyriform parasites* (fig. 4) are neither numerous nor typical of *T. parva*. Intermediate forms between pyriforms and ovoids (figs. 23, 24, 36, 42) are often encountered. These and the pyriforms probably belong in the same category as do parasites from which a single pseudopodium-like process (figs. 11, 12) is protruding. Judging from observations on the living parasites (*Parasitology*, vol. II. p. 334) the movements of such forms are slow. In the parasite depicted in fig. 12 the chromatin mass is curved dumb-bell shaped.

Forms apparently undergoing division into two:—We have already referred to appearances observed in the chromatin masses of rounded, ovoid, bacilliform and amoeboid parasites which suggest that these may be undergoing nuclear division. In some parasites two distinct masses of chromatin are observable lying at opposite poles of the parasite. This is clearly seen in some oval (fig. 32) and bacilliform parasites (fig. 27). In most bacilliforms showing two distal masses of chromatin, the rod-shaped body of the parasite is bent at an angle (figs. 17, 18, 19, 20, 30, 39, 44) midway along its length. We have frequently observed such parasites alive; they present a curved dumb-bell-shaped appearance (*Parasitology*, vol. II. p. 336, Diagram 3), but we have not as yet been able to see them divide. The appearances seen in the stained parasites suggest that they are dividing forms. In one case (fig. 19) a small mass or strand of chromatin was observed at the bend. As will be seen by reference to the plate, some of these "bent" forms are very much larger than others. Bent dumb-bell-shaped masses of chromatin may be observed in minute pear-shaped parasites (fig. 35) as well as in large ones (figs. 12, 34, 42). If these appearances observed in the chromatin denote division, then we must conclude that both large and small parasites may undergo nuclear division in a similar manner.

Forms apparently undergoing division into four:—In the blood films taken, especially in the later stages of the disease, we not infrequently encountered parasites containing four masses of chromatin lying in distinctly blue-staining cytoplasm (fig. 47), or apparently projecting (fig. 48) from a small mass of indistinctly staining cytoplasm. These may be referred to as true "cross-forms." Fig. 31 perhaps represents a parasite which has broken away from such a cross-form, three masses of chromatin being left in the remaining cytoplasm. At times, more particularly immediately before the death of the host, irregularly formed, possibly amoeboid, parasites were seen which contained three (fig. 46; note vacuoles) or four (fig. 45) masses of chromatin.

Free parasites were rarely encountered in stained films; those that were observed were ovoid or bacilliform. We have occasionally observed them in fresh blood, as noted elsewhere (*Parasitology*, vol. II. p. 338).

The relative numbers of different types of parasites.

In the following tables we give the results of some counts in which we attempted to classify the parasites according to types. For this purpose we usually examined 250 corpuscles in each film and noted the kinds and numbers of parasites which occurred in them. We do not include the rarer types of parasites in the tables. The numbers beneath the bracketed signs indicate, in round figures, the percentages of different parasites present in the peripheral blood on succeeding days. It was soon found to be very difficult to classify parasites under the various types, since intermediate forms (for instance between bacilliforms and comma-forms) were encountered; nevertheless, an arbitrary division was made, and the results appear worth recording.

In the signs used in the tables the brackets ( ) represent corpuscles which include the parasites. Thus:

The signs	stand for a corpuscle harbouring
(O)	1 round or ovoid parasite.
(,)	1 drum-stick or comma-shaped parasite.
(I)	1 rod-shaped parasite.
(c)	1 thick bent sausage-shaped parasite.
(OO) to (III)	2 to 3 rounded bacilliform etc. parasites of the types described under the preceding signs.
(3)	Groups such as (OOI). (OO,), (OII), (,II).
(O_4), (I_4)	4 rounded or rod-shaped parasites.
(5–6)	5–6 parasites of various types.

The day of illness is reckoned from the time when the pathogenic ticks were placed upon the cows.

Cow I.

Day	(O)	(,)	(I)	(c)	(OO)	(,,)	(II)	(O,)	(IO)	(I,)	(OOO)	(,,,)	(III)	(3)	(O_4)	(I_4)	(5-6)
19	71	18	2	1	4	1	0	1	1	0	0	0	0	0	0	0	0
20	66	16	8	0	6	0	−1*	0	1	−1	1	0	0	1	0	0	0
21	26	48	9	0	2	6	2	−1	−1	−1	0	1	0	0	0	0	0
22	28	38	13	3	4	3	3	1	−1	−1	1	1	−1	0	−1	0	0
23	60	11	13	0	6	0	4	0	0	3	1	0	−1	2	−1	0	1
24	25	28	24	3	1	4	4	2	2	2	2	2	0	0	−1	0	0
25	37	25	3	0	10	6	1	3	1	−1	3	4	0	0	2	0	−1
26	23	9	28	0	4	8	8	2	2	2	0	4	2	0	2	0	2
27	20	10	20	0	3	6	9	2	6	3	2	−1	6	0	3	0	−1
28	6	5	41	5	2	4	17	−1	4	1	3	−1	4	2	3	0	1
29	16	17	20	0	6	8	7	1	4	2	2	1	7	0	5	0	−1
30	26	12	4	0	17	6	4	3	1	1	8	6	1	0	5	0	3
31	31	4	11	0	17	1	10	1	3	0	9	−1	2	1	1	4	−1

Cow II.

21	63	18	11	0	2	1	1	0	0	2	−1	0	0	0	−1	0	−1
22	50	16	22	0	4	1	3	−1	3	−1	0	0	0	0	−1	0	0
23	43	27	16	0	3	2	1	2	−1	2	0	0	0	0	−1	0	0
24	42	19	19	0	10	4	1	1	2	−1	1	0	0	−1	−1	0	0
25	30	16	18	0	8	4	4	1	1	1	2	−1	3	0	−1	0	1
26	22	28	22	5	−1	12	2	2	0	2	2	0	0	0	−1	0	0
27	58	13	9	0	7	3	2	3	1	−1	−1	0	0	3	0	0	0
28	12	21	30	3	1	7	5	0	2	4	8	0	1	0	3	0	0
29	47	15	6	0	12	3	5	2	4	0	2	0	2	2	0	−1	0

* The numbers "−1" denote less than 1%.

It is clear that the rounded, the comma-shaped or clubbed and the rod-like forms represent the majority of the parasites seen in stained films, the rounded forms usually predominating. The rod-shaped forms regarded as a whole, that is when occurring singly, in pairs and larger numbers, or with other types of parasites, appear in larger numbers as the disease progresses, their number falling towards the end of the malady.

The increase of parasites observed during the course of the disease.

We have already recorded elsewhere (*Parasitology*, vol. II. p. 327) the steady increase in the percentage of infected corpuscles which was observed daily in the two cows up to the fatal termination of the disease. We desire to add some particulars here showing that an increase takes place in the number of corpuscles containing two or more parasites. We have tabulated (Tables I and II) our results and have represented them in graphic form in Charts I and II.

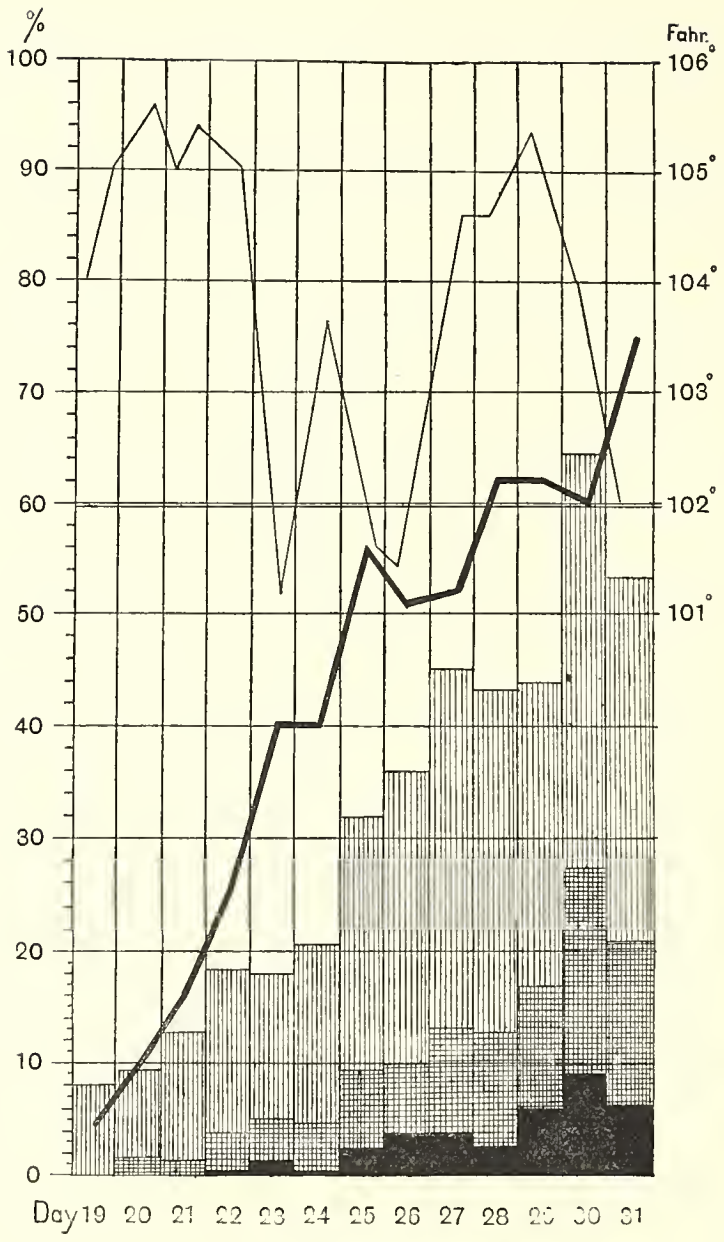

Chart I (relating to Cow I).

The body temperature is indicated by the thin line above. The increase in the percentage of infected corpuscles is represented by the bold black line rising from 5 % to 75 %. The vertical columns represent the total number of infected corpuscles taken as 100; the white parts of the columns represent the proportion of corpuscles containing a single parasite, the vertically shaded, cross-shaded and black parts indicate respectively the proportion of corpuscles containing two, three, and four or more parasites. The numbers (19—31) below indicate the days reckoned from the time when the infected ticks were placed on the cow.

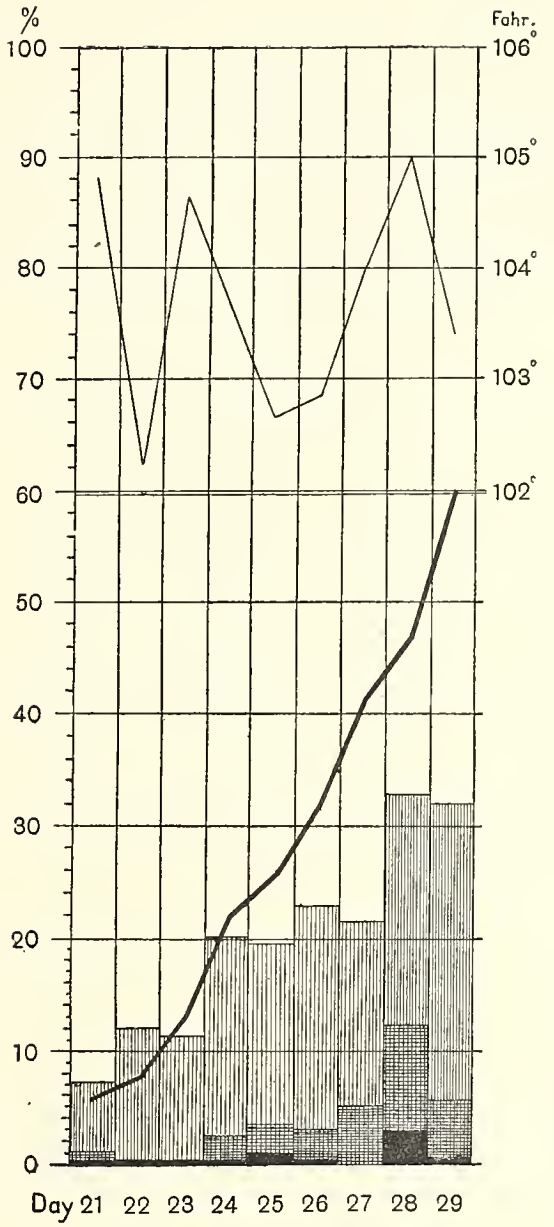

Chart II (relating to Cow II).

For explanation refer to legend appended to Chart I.

TABLES I & II.

Showing the percentage of infected red blood corpuscles and the percentage of such corpuscles which contained one, two, three and four or more parasites, commencing on the day when the parasites were first detected in the peripheral blood and ending on the day preceding that on which the cows died. These counts served for the construction of the accompanying charts. The percentage of infected corpuscles was usually determined by counting 250 consecutive corpuscles in the central portion of each blood film. The percentages of infected corpuscles containing different numbers of parasites are based upon the examination of 100 such corpuscles. The day on which the parasites appeared in the animal's blood is reckoned from the day on which it was infested with pathogenic ticks (*Rhipicephalus evertsi*).

TABLE I. (*Cow I.*)

Day	% r.b.c. infected	% r.b.c. containing 1	2	3	4 or more parasites
19	5	92	8	•	•
20	10	90·8	7·6	1·6	•
21	16	87·6	11·2	1·2	•
22	25	82·4	14·4	2·8	·4
23	28	82·4	12·8	3·6	1·2
24	40	80	15·6	4	·4
25	56	68·4	22·4	6·8	2·4
26	51	64·4	26	6	3·6
27	52	55·2	32	9·2	3·6
28	62	57·2	30·4	10	2·4
29	62	56	27·2	11·2	5·6
30	60	36	37	18·2	8·8
31	75	47·2	32·4	14·4	·6
32	Cow died.				

TABLE II. (*Cow II.*)

21	6	92·8	6	·8	·4
22	8	88	11·6	•	·4
23	13	88·8	10·8	•	·4
24	22	80	17·6	2	·4
25	26	80·4	16	2·6	1
26	32	77·6	19·6	2·4	·4
27	41	80·4	16·4	3·2	•
28	47	67·6	20·4	9·2	2·8
29	60	68·5	26	5	·5

The distribution of the parasites in the blood from various organs.

An examination of the blood corpuscles in smears made from various organs immediately after death gave a remarkably uniform result with regard to the percentage of infected corpuscles and the relative numbers

of corpuscles containing different numbers of parasites. The results are given in the following Table:

TABLE III.

Showing the percentages of infected r.b.c. and the relative numbers of infected r.b.c. containing one to eight parasites. The percentages are based on a count of 1000 r.b.c. made from each preparation excepting in the case of brain and marrow smears where, owing to few r.b.c. being present, only 500 corpuscles were counted. The blood films and organ smears were made immediately after the death of Cow I.

	Blood from jugular	Heart	Lung	Liver	Spleen	Kidney	Supra-renal	Brain	Bone marrow
% infd. r.b.c.	**73·3**	**69·7**	**71·2**	**74·8**	**72·8**	**75·9**	**75·6**	**73·2**	**84·4** *
r.b.c. containing									
1 parasite	28·2	25·1	31·4	29	27	29·5	28·8	31·8	33
2 parasites	29·1	25·8	24·3	27	27	26·8	30·5	23·8	29·2
3 ,,	9·4	12·6	9·1	11	9·3	11	9·2	11·6	12
4 ,,	5·4	4·9	4·8	5·7	6·4	6·6	5	4	6·6
5 ,,	7	1·9	1·3	1	1·6	·9	1·1	1·4	1·6
6 ,,	5	·9	·3	·8	1·2	·7	·6	0	1·2
7 ,,	0	·4	0	·2	·2	·2	·2	0	·2
8 ,,	0	·1	0	·1	·1	·2	·2	·6	·6

* This count is probably too high owing to the pale staining of many uninfected r.b.c. which may consequently have been overlooked.

Conclusions.

(1) The best results in the staining of these minute parasites are obtained by the ordinary method of drying the blood films, fixing them in absolute alcohol and staining them according to Giemsa. Methods of wet fixation give poor results, because the corpuscles do not become flattened as in drying, the consequence being that the haemoglobin clouds the image of the parasites.

(2) The percentage of infected corpuscles in the peripheral circulation rises steadily as the disease progresses and at the same time there is a progressive increase in the proportion of infected corpuscles which contain two, three and four or more parasites.

(3) The percentage of infected corpuscles observed in smears from various internal organs immediately after death, and the percentage of corpuscles containing more than one parasite, coincide with those obtained in the case of the peripheral blood. In other words, there does not appear to be a heavier infection of the corpuscles in any of the internal organs than there is in the general circulation.

(4) Free parasites are rarely encountered in the blood.

(5) Whilst the parasite is very pleomorphic, the commonest forms seen in stained preparations are ovoid or rounded and comma-shaped or clubbed. The proportion of bacilliform parasites is also fairly large and increases as the disease advances but may fall toward the end of the malady. Parasites having a pyriform shape are rare, and observations on living parasites indicate that they simply arise in the course of transitory or amoeboid movements; consequently such parasites are not to be compared with the common and typical pyriforms in *Piroplasma*, the latter, when formed, maintaining their shape for a considerable time. In other words, the pyriforms in *Piroplasma* represent a definite stage in the life history of the parasite, whereas in *Theileria* they do not. The study of stained and living *Theileria* confirms us in the opinion already expressed (*Parasitology*, vol. II. p. 326) that this parasite deserves generic rank since it differs in essential characters from *Piroplasma*.

(6) Judging from the study of stained specimens, the observer is tempted to conclude that the prevalent type of small ovoid parasite grows in size and becomes pleomorphic. The chromatin mass in ovoid parasites may become elongated or horse-shoe-shaped (figs. 9, 24, 36, 42), and divides directly into two masses by a median constriction (figs. 10, 12, 34, 35), the masses subsequently moving to opposite poles of the parasite (figs. 22, 32, 45). In bacilliform parasites the chromatin mass simply divides (figs. 19, 28, 30, 39) and the two masses wander to opposite poles of the parasite (figs. 27, 29, 30); in many cases the long parasite shows a marked bend at the point where the final division of the cytoplasm of the daughter cells is to follow (figs. 17—20, 30, 39, 44). Finally, in certain parasites which are rich in chromatin, the mass may undergo division into four giving rise to "cross-forms" (figs. 47, 48) the subsequent separation of the cytoplasm then liberating the daughter organisms (fig. 31). We have not observed more than eight parasites within an infected corpuscle.

(7) Observations on living parasites (*Parasitology*, vol. II. p. 335, Diagram 2) have established the fact that oval parasites may, when active, protrude processes, become constricted, or assume a pyriform shape. In some cases (vol. II. p. 336, Diagram 3, B and C) we observed the formation of bent dumb-bell-shaped parasites from ovoid or short rod-like forms. We were never able, however, to observe actual division in living parasites. Therefore we were led to doubt if multiplication of intracorpuscular parasites actually takes place (p. 334), and assumed that if it takes place the process "must be very slow." The careful study of

stained films suggests that intracorpuscular division may take place, consequently further observations on the living parasite will have to be made before reaching a final conclusion. We may note however that observations on *Theileria* are fraught with considerable difficulty owing to the minuteness of the parasite.

Correction.

In our previous paper (*Parasitology*, vol. II. p. 327) we stated that Theiler (1904) was the first to demonstrate that ticks transmitted East Coast Fever, overlooking at the time the earlier work of Lounsbury to whom the credit is due. Lounsbury reported upon positive experiments more especially with *Rhipicephalus appendiculatus*, in his *Report of the Government Entomologist* (Cape of Good Hope) for the years 1902, 1903 and 1904.

It appears moreover that the first to distinguish East Coast Fever from Redwater was the late Dr Hutcheon (*Transvaal Agricult. Journ.* April 1903, p. 45). A correspondent in South Africa writes of Dr Hutcheon: "He was such a kindly soul that he preferred to let Dr Koch say definitely that they were distinct seeing that the latter had confused them in East Africa." To state the matter accurately then, the credit of first distinguishing East Coast Fever from Redwater is due to Hutcheon in the first place and in the second place to Koch and to Theiler.

G. H. F. N.

EXPLANATION OF PLATE XII.

All the figures were outlined with an Abbé-Zeiss camera lucida, using a 2 mm. apochromatic homogeneous immersion objective, and compensating ocular 18 (Zeiss). The magnification is approximately 3500 diameters. Blood of Cow I.

Figs. 1, 2. Ovoid parasites, with clear area (? vacuole) and chromatin at one end. Days 19—22.

Fig. 3. Small pyriform parasite, chromatin at broad end. Day 20.

Fig. 4. Large pyriform parasite. Day 31.

Figs. 5, 6. Comma-shaped parasites, with large chromatin mass at one end. Days 19, 24.

Figs. 7, 8. Short, broad, rod-like forms, with large terminal masses of chromatin. Days 24, 25.

Fig. 9. Rounder parasite, with chromatin cap at one end, and clear area. Day 25.

Fig. 10. Rounder parasite, with horse-shoe-shaped mass of chromatin; slight concentration of chromatin at the ends of the horse-shoe. Days 23, 25.

Fig. 11. Large parasite with pseudopodium and chromatin at one end. Day 24.

Fig. 12. Parasite with pseudopodium and chromatin mass in shape of a bent bar, with thickenings at either end. Day 26.

Fig. 13. Rod-shaped parasite with terminal mass of chromatin. Day 29.

Fig. 14. Shorter, rod-like parasite with terminal mass of chromatin. Day 21.

Fig. 15. Slightly bent rod or elongate comma, chromatin terminal. Day 26.

Fig. 16. Slightly bent rod, chromatin mass not quite terminal. Day 28.

Figs. 17—20. Bent, dumb-bell-shaped parasites, apparently dividing forms.

Fig. 17. Parasite with large chromatin masses. Day 24.

Fig. 18. Smaller parasite. Day 21.

Fig. 19. Parasite with terminal chromatin masses and a third, very small, median chromatin mass. Day 22.

Fig. 20. Parasite with one end somewhat irregular. Day 25.

Figs. 21—31 represent infected blood corpuscles each containing *two* parasites.

Fig. 21. Two ovoid parasites, each with chromatin at one end. In the largest parasite the chromatin mass shows signs of division, being slightly concentrated at the ends of the cap. Day 31.

Fig. 22. One parasite (reniform) has two chromatin masses arranged terminally, the other parasite represented is somewhat comma-shaped. Day 27.

Fig. 23. One parasite is ovoid, the other arcuate with central mass of chromatin showing a more deeply staining centre. Day 24.

Fig. 24. Ovoid parasite and short rod. The chromatin mass of each parasite is large. Day 24.

Fig. 25. Two rod-like parasites, each with enlarged end containing the chromatin mass. Day 26.

Fig. 26. Rod-like parasites, one long and slightly curved. Day 28.

Fig. 27. Rod-shaped parasites, one with two terminal chromatin masses, the other curved like a long attenuate comma. Day 27.

Fig. 28. Rod-shaped parasites, each with a slightly irregular, lobed (dividing?) mass of chromatin. Day 29.

Fig. 29. Two rod-like parasites, lying across each other, forming an apparent "cross-form." Day 21.

Fig. 30. Bent dumb-bell-shaped and very short rod-shaped parasites. The short rod-like parasite is composed almost entirely of a lobed mass of chromatin. Day 29.

Fig. 31. Small comma-shaped parasite, together with an irregular parasite showing three masses of chromatin. Breaking up of a cross-form? Day 26.

Figs. 32—35 represent *three* parasites contained in each of the infected blood corpuscles.

Fig. 32. Two ovoid parasites and one comma-shaped. One ovoid parasite shows two well-marked terminal masses of chromatin. Day 24.

Fig. 33 represents two comma-shaped and one ovoid parasite. Day 27.

Fig. 34. One ovoid, slightly pyriform parasite, with chromatin cap showing apparent division; together with two elongate comma-shaped parasites. Day 31.

Fig. 35 represents three small parasites, one with horse-shoe-shaped cap of chromatin showing thickenings at either end. Day 25.

Figs. 36—38 represent *four* parasites in each infected blood corpuscle. Nearly all the parasites are more or less rod-like or comma-shaped. Days 26, 28.

Fig. 39 represents three parasites in a corpuscle; one of the parasites is apparently dividing. Day 30.

Fig. 40 represents three rod-like parasites and one small pyriform. Two of the rod-shaped parasites are arranged across each other (apparent "cross-form"). Day 30.

Fig. 41 represents three parasites, two arranged as an apparent "cross-form," the other is elongate comma-shaped. Day 26.

Fig. 42 represents *six* parasites within one blood corpuscle—the parasites are chiefly ovoid or comma-shaped. Day 31.

Fig. 43 represents *five* parasites in a corpuscle. Two of the parasites are rod-like, each with two terminal masses of chromatin and lying diagonally across each other. Day 31.

Fig. 44 represents *seven* intracorpuscular parasites, five of them small. Day 31.

Fig. 45 represents three parasites in a corpuscle. One of the parasites appears amoeboid and contains four minute chromatin masses. Day 32.

Fig. 46 shows a parasite with three chromatin masses, connected by thin protoplasmic strands, probably with vacuoles between. Day 29.

Fig. 47. True cross-form. Day 30.

Fig. 48 represents a corpuscle containing three parasites—one cross-form, one rod-like and one ovoid showing horse-shoe-shaped cap of chromatin with thickenings terminally. Day 31.

A REVISION OF THE BRITISH LEECHES.

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(Plates XIII to XV and 16 Text Figures.)

CONTENTS.

Introduction.

Since the appearance of Johnston's *Catalogue of the British Non-parasitical Worms in the Collection of the British Museum*—a work which although not published until 1865 had been completed some ten years earlier and consequently embodies observations made more than half a century ago—the Hirudinea of the British Islands have been largely neglected. Apart from this the interest now being taken in leeches by students of their protozoan parasites and the absence of any recent work upon this group of our fauna seems sufficient apology for the revised descriptive catalogue attempted in the following pages.

The material upon which these observations are based has been collected, as far as the fresh-water species are concerned, for the most part in the neighbourhood of Cambridge. Consignments of leeches however have been received from other quarters, and in this connection my thanks are due, amongst others, to Mr Thomas Edwards for specimens of *Piscicola* from the River Test, in Hampshire; to Dr F. W. Gamble for examples of *Trocheta* from the Withington Sewage Works, near Manchester; to Mr Hugh Stowell and Mr C. H. Ball for further material from the same source, and to Mr M. R. Pryor for special information regarding the occurrence of this species in England

most readily placed at my disposal. Finally I desire to express my thanks to Mr Edwin Wilson, of Cambridge, who has spared no pains to make the coloured illustrations accompanying this memoir exact representations of the living examples placed before him.

With regard to the number of the British leeches, it may be stated of the fresh-water forms with some degree of certainty that ten species now occur in these Islands. An eleventh species, *Hirudo medicinalis*, is included in the following list, although there is very little doubt that it no longer occurs in the wild state. We cannot speak so positively regarding the number of marine species. Our knowledge of the marine Ichthyobdellidae leaves much to be desired and few European species are well established. *Pontobdella* alone is frequent upon our shores; *Branchellion* has been noted twice, and there remain a number of forms which have been recorded from time to time, chiefly from the coast of Scotland, the descriptions of which are generally not sufficiently adequate to enable us to do more than guess at the species upon which they have been based. Some of these have been referred provisionally to *Trachelobdella lubrica* (Grube, 1840) and it seems not improbable that at least one other species has been observed in British waters in addition to the three marine leeches already noted.

The descriptions here given apply to features which may be seen either by the naked eye or with the assistance of a good dissecting lens. The form described, unless otherwise stated, is that assumed by the leech when in a moderate or average state of extension. The treatment of the various morphological features indicated in the series of figures supplementing the coloured illustrations is purely schematic.

Whilst every endeavour has been made to make the synonymical tables as complete as possible, the accompanying bibliographical references make no pretence of being exhaustive.

The Classification here adopted is that laid down by Professor Raphael Blanchard in a well-known monograph on the leeches of Italy (1894) and subsequently modified by him in other works. The same authority also has generally been followed with respect to the synonymy of the species referred to.

Diagnostic Characters.

In the leech, as is well known, the number of rings exceeds the number of somites or segments into which the body is divided, and throughout the greater part of its length these rings resolve them-

selves into a series of regularly recurring groups corresponding to the successive somites of the body.

Towards the ends of the body the number of rings in a group becomes smaller, and we frequently find at the extremities one or more somites represented respectively by only one ring.

For the sake of brevity the ring or group of rings corresponding to the metameric divisions of the body are themselves alluded to as somites (segments, zoonites), and for each of the similar groups containing an equal number of rings, which occur throughout the greater part of the body of every leech, the term *complete somite* has been retained.

In the number of rings of which a complete somite is composed, in the number of such complete somites, in short, in an analysis of the external metamerism, we have characters of the greatest importance in the determination of genera and species.

In the following descriptions I have not adopted the neuromeric standard of somite limits advocated by Moore (1900) and Castle (1900 b) and since supported by Livanow but, as far as the delimitation of somites is concerned, have adhered to the original conception of Gratiolet (1862) which has subsequently been elaborated and supported by writers of such divergent opinions as Whitman and Blanchard on the one hand and Apáthy on the other. The first ring of the complete somite, as here understood, is the sensory ring which lodges a ganglion of the ventral chain and bears externally the "metameric sensillae" (Whitman) often rendered conspicuous by association with special colour markings and by elevation upon more or less prominent papillae. It has long been recognised that a somite is determined by the presence of a ganglion and that in the central nervous system we have twenty-one free single ganglia with a mass of fused ganglia at either extremity. Apáthy (1888 b) found six ganglia in the circumpharyngeal ganglionic mass, whilst in the posterior ganglionic mass Whitman (1892) found seven; and these results have been confirmed by subsequent workers.

Thus there are 34 ganglia and somites in the body of the leech. Of these somites, seven are absorbed by the posterior sucker and consequently we have to account for 27 in the rest of the body. The first four somites and sometimes part of the fifth may be involved in the formation of the anterior sucker, and the genital apertures, as Apáthy first pointed out, invariably occur, the male in somite XI and the female in somite XII. This rule holds good even in the case of

Helobdella stagnalis, where the male and female genital apertures still emerge from their respective somites although opening by a common orifice between them.

With regard to the sense organs, we are only concerned with the "metameric sensillae" already referred to, which are confined to the first or sensory ring of each somite and occur in strict series in definite longitudinal lines. In describing these lines the nomenclature adopted is that suggested by Livanow (1904) which again is but a modification of that given by Apáthy. These lines occur dorsally and ventrally in pairs with respect to a median line, and counting from the median line outwards we get (1) an inner and (2) an outer paramedian pair; (3) an intermediate pair; (4) an inner and (5) an outer paramarginal pair. Finally (6) a marginal pair coincide with the edges of the body. Ventrally the outer paramedian lines are slightly nearer together than dorsally. Sensillae are not present on all these lines in every species.

By noting the position of any colour spot, papilla or other external feature with regard to its situation transversely on any particular ring and longitudinally on one of the above lines, we are able to locate precisely as it were its latitude and longitude on the surface of the body.

The remaining diagnostic characters call for no special explanation and we may now proceed to a consideration of the species enumerated on pp. 186—187.

Sub-order I. RHYNCHOBDELLAE.

Marine and fresh-water Hirudinea with colourless blood, with an exsertile proboscis, without jaws.

Family I. **ICHTHYOBDELLIDAE.**

Body cylindrical or flattened, formed of two distinct regions, (i) *a short narrow anterior portion or "neck" which includes the clitellum with the genital orifices and* (ii) *a long, large posterior region or "abdomen." The anterior as well as the posterior sucker is a permanent cupuliform or discoid organ distinct from the body. Eggs included in chitinous capsules which are attached to foreign bodies.*

Genus: **Branchellion,** *Savigny,* 1822.

Synonymy:

Branchiobdella, de Blainville, 1827 (not *Branchiobdella*, Odier, 1819). *Branchellia*, Gervais, 1845.

Marine leeches, ectoparasitic on fish. Body flattened, the posterior region with lateral, paired, foliaceous, non-digitate branchiae. Suckers cupuliform, excentrically attached, the posterior very large and studded upon its inner surface with numerous, small subsidiary suckers. Complete somite formed of three rings.

This genus is represented in European waters by a single species (Blanchard, 1894 a, p. 85).

Branchellion torpedinis, *Savigny*, 1822.

Plate XIII, Figs. 1, 2, 3. Text Fig. 1.

Synonymy and Literature:

Branchellion torpedinis, Savigny, 1822, p. 109 ; Risso, 1826, p. 432 ; Savigny, 1826, p. 451 ; Moquin-Tandon, 1826, p. 141 ; Milne-Edwards, in Lamarck, 1835, p. 529 ; Cuvier, 1836, p. 51, pl. xxiii, fig. 3 ; Moquin-Tandon, 1846, p. 282, pl. i, figs. 1—10 (coloured) ; Leydig, 1851, p. 315, pl. ix, fig. 1 (anatomy of branchia) ; Grube, 1851, p. 108 ; Johnston, 1865, p. 38 (recorded from England) ; Apáthy, 1888 a, p. 153, etc., pl. viii, fig. 1 (diagram of annulation, etc.), and fig. 11 (details of branchiae) ; R. Blanchard, 1894 a, p. 85 ; R. Blanchard, 1894 b, p. 11 ; Apáthy, 1901 a, p. 211 (neurofibrillae) ; Apáthy, 1901 b, p. 707 (histology of light sensory cells) ; Pérez and Gendre, 1904 a, p. 113 (muscle fibres) ; *ibid.* 1904 b, p. 605 (ovogenesis) ; Pérez, 1906, p. 447 ; Holt, 1907, p. 102 (recorded from Ireland).

Hirudo (*Branchiobdella*) *rudolphii*, de Blainville, 1827, p. 241.

Branchiobdella rudolphii, Diesing, 1850, p. 443 ; Polonio, 1863.

Branchiobdella torpedinis, de Blainville, 1828, p. 556, pl. xxxiv, figs. 1, 1 a and 1 b.

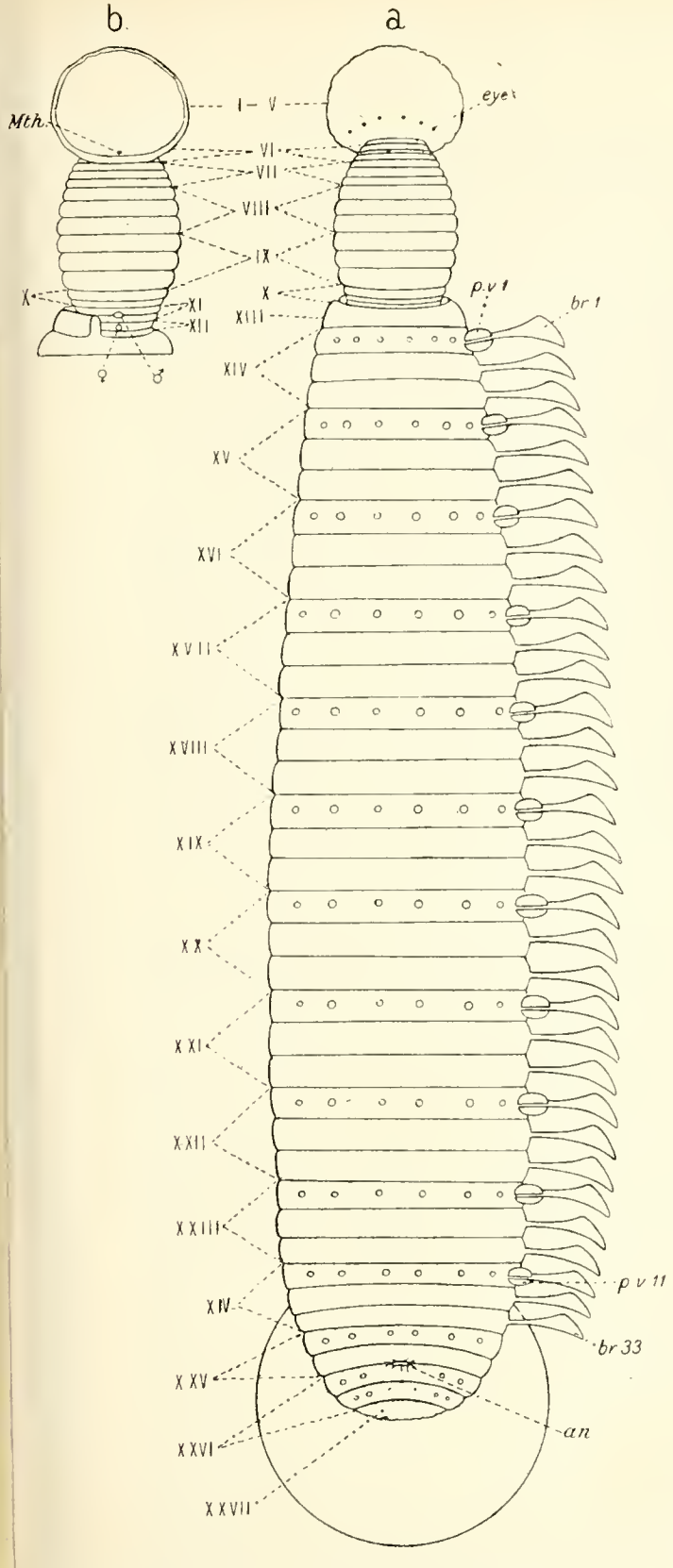
Hirudo (*Branchiobdella*) *torpedinis*, Gervais, 1836, p. 627, pl. ccxi, fig. 1.

Branchellia torpedinis, Gervais, 1845, p. 573.

Branchellion orbinensis, de Quatrefages, 1852, p. 279, pl. vi, fig. 1 and figs. 2—13 (anatomy).

Branchellion rhombi, van Beneden and Hesse, 1863, p. 33, pl. ii, figs. 17—21 (bad).

Diagnosis. The two regions of the body are sharply defined. The anterior is partly invaginated into the posterior region, the first ring of which forms a fold surrounding and overlapping the posterior half of the clitellum. [Apáthy (1888 a, p. 170) has pointed out that this fold is really composed of two rings, one lying upon its outer and one upon its inner surface. These two rings, which form somite XIII, lie one in front of the other in very young individuals and are gradually gathered up into a "preputial fold" as maturity is approached.] Colour brownish black, with six longitudinal series of yellowish white spots on the dorsal and four on the ventral surface ; the spots occur on the first ring of each



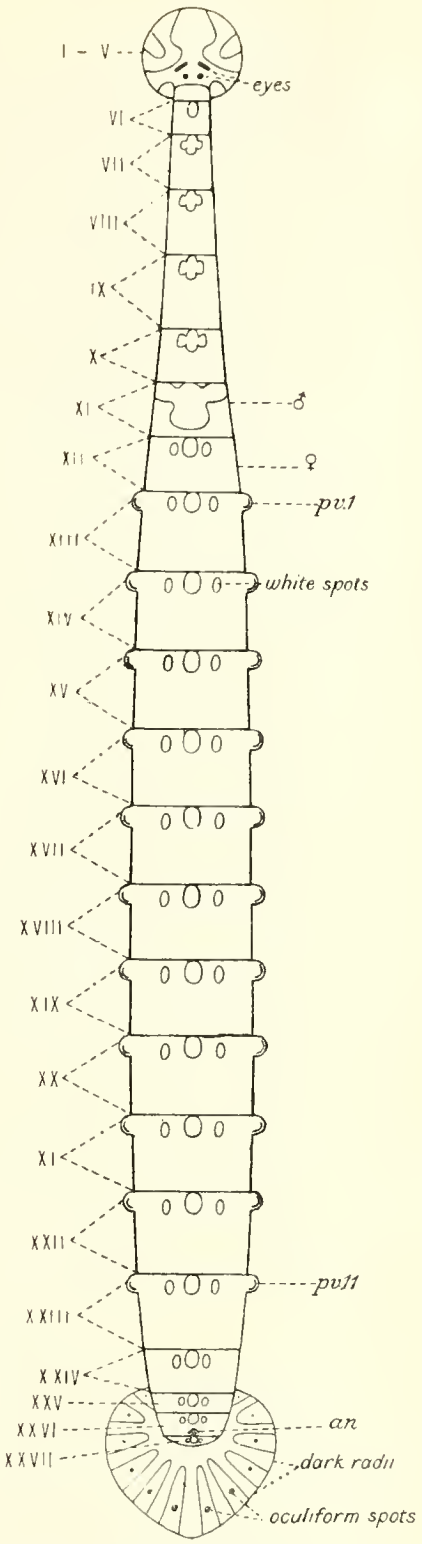


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Fig. 1. *Branchellion torpedinis.*

Fig. 2. *Piscicola geometra.*

Fig. 1. *Branchellion torpedinis.* (*a*) Diagram showing annulation on dorsal surface, position of branchiae and other external features. Branchiae emitted on left side of body for the sake of clearness. *br.* 1, *br.* 33, branchiae of the first and thirty-third pairs. *Mth.* Mouth. *an.* Anus. *pv.* 1, *pv.* 11, pulsating vesicles of the first and eleventh pairs. Somites indicated in Roman numerals. (*b*) Diagram showing ventral surface of anterior extremity; the "preputial fold" cut away so as to show genital apertures. (Founded on a diagram by Apáthy.)

Fig. 2. *Piscicola geometra.* Diagram showing disposition of somites (in Roman numerals), pulsating vesicles (*pv.* 1 to *pv.* 11), white spots, etc. (See p. 140.)

somite. Or the colour may be of a roseate hue which fades in alcohol. (R. Blanchard.)

Somites XIV—XXIV in the posterior region of the body are complete with three rings. Each of the 33 rings composing these eleven somites carries a pair of lateral, foliaceous, crispate branchiae. A pulsating vesicle is situated at the base of each of the branchiae arising from the first ring of each somite. Six eyes, disposed in a transverse curved line, on the dorsal surface of the anterior sucker, in somite V (Apáthy). The male genital orifice is situated between the two rings of somite XI; the female orifice lies upon the first ring of somite XII; somites X, XI and XII, each composed of two rings, form the clitellum. The anus lies between somites XXV and XXVI.

Length 30—50 mm.; width 8—16 mm. including the branchiae.

[The following measurements are taken from an example in alcohol, from Naples, in the possession of the University Museum of Zoology, Cambridge:—Length 49 mm., width of body 11 mm., total width including branchiae 16 mm.; diameter of anterior sucker 3·5 mm., of posterior sucker 9 mm.]

Distribution, Hosts. *B. torpedinis* is parasitic chiefly upon the electric rays (*Torpedo*). Blanchard records it from a wrasse (*Labrus* sp.) and from *Rhinobatus thouin*; van Beneden and Hesse found it on a turbot (*Psetta* [*Rhombus*] *maxima*) and its occurrence on *Raia clavata* is noted below. It is found in the Mediterranean, and in the Atlantic where its range extends along the West coast of Africa as far as Senegal (Blanchard). In British waters it has been recorded twice. (1) Johnston (1865, p. 39) catalogues an English example (precise locality unknown) taken "with soles," and (2) Holt (1899, p. 4) records a single individual taken from the pelvic fin of a thornback (*Raia clavata*) in Blacksod Bay on the west coast of Ireland.

Genus: **Trachelobdella,** *Diesing,* 1850.

Synonymy:

Calliobdella, van Beneden and Hesse, 1864. *Callobdella*, R. Blanchard, 1894.

Ectoparasitic on marine fish. Without eyes. Anterior sucker reduced. Posterior region of the body cylindrical or flattened in young individuals, ventricose in adults, with paired lateral pulsating vesicles. Complete somite composed of six rings, formed by the more or less distinct subdivision of three primitive rings.

That this genus is represented in British waters is beyond dispute; we are unable however to state positively to which species of *Trachelobdella* the examples recorded from our coasts are to be referred.

Dalyell (1853) describes two marine leeches in addition to *Pontobdella*. Of one of these, which he calls *Hirudo campanulata*, he had but two specimens and his description and drawings are altogether inadequate; of the other, which he obtained in considerable numbers, we have a detailed description and fairly good figures. In this latter species, which he describes as *Hirudo vittata*, a name originally proposed by Chamisso and Eysenhardt (1821), the two regions of the body are well defined, the posterior region bears paired lateral pulsating vesicles, there are no eyes and we have clearly an example of *Trachelobdella*.

Johnston (1845) under the name *Piscicola marina*, which in his *Catalogue* of British species (1865) is changed to *Pontobdella littoralis*, gives an indifferent description of a leech parasitic on *Aspidophorus cataphractus* from the coast of Northumberland, with which he considers Dalyell's *Hirudo vittata* to be synonymous. To Johnston's species Thompson (1856, p. 426) refers three leeches from the Irish coast taken respectively from *Lophius* sp., from a halibut and from a cod, and M'Intosh (1875, p. 114, pl. 5, figs. 3—6) ascribes a form, of which he gives coloured figures, said to be not uncommon on *Cottus bubalis*, at St Andrews.

In 1864, van Beneden and Hesse described three species of *Calliobdella* (= *Trachelobdella*), viz :—*C. lophii*, parasitic on *Lophius piscatorius*, *C. punctata* parasitic on *Cottus bubalis*, and *C. striata*. Insufficient and inaccurate as the descriptions and figures are, it is evident that *Hirudo vittata* and *C. lophii* are synonymous and that *Pontobdella littoralis* has affinities with the other two forms.

Finally Scott (1901, p. 138) records from the coast of Scotland a leech which he describes as *Trachelobdella lophii*, found "In the gill pouches of the angler-fish, *Lophius piscatorius*, captured in the Firth of Forth (1894), and in the Moray Firth (1899)."

From the above evidence it is clear that *Trachelobdella* is represented upon our coasts, and that in more than one instance we have records of a form which appears to be identical with the *Trachelobdella* (*Calliobdella*) *lophii* of van Beneden and Hesse.

Whether or not *T. lophii* is a good species remains to be considered.

Apáthy (1888 c) unhesitatingly refers *C. lophii*, *C. punctata*, *C. striata*, *Pontobdella littoralis* (Johnston) and perhaps *H. campanulata* (Dalyell) to one species, *Calliobdella lubrica* (Grube), of which he

gives a diagnosis based on abundant living material. R. Blanchard (1894 b), who establishes the identity of the genus *Callobdella* with *Trachelobdella* (Diesing, 1850) largely supports this view, amending the specific characters of *T. lubrica* and including among its synonyms *C. punctata*, *C. striata* and *P. littoralis*. Concerning *C. lophii* he writes, "La validité spécifique...nous semble...très douteuse,...il sera probablement nécessaire de la réunir un jour soit à l'espèce précédente (*T. lubrica*) soit à la *P. campanulata* Dalyell, si tant est que celle-ci constitue une espèce solidement établie."

Johansson, on the other hand, in a work on the Swedish Ichthyobdellidae (1898, pp. 672—5) denies even that *Callobdella* and *Trachelobdella* are synonymous. His observations which, as far as *Callobdella* is concerned, are based upon material preserved in alcohol, are far from convincing. Of two facts at least we may be certain. *Trachelobdella* and *Callobdella* are synonymous, and only one European species is at present firmly established, namely *T. lubrica* (Grubé, 1840).

In the present state of our knowledge, therefore, and until the examination of living material can be carried out, we are compelled provisionally to refer to this species the examples of *Trachelobdella* recorded from our coasts.

Trachelobdella lubrica, *Grube*, 1840.

Synonymy and Literature:

Pontobdella lubrica, Grube, 1840, p. 60.
Piscicola marina, Johnston, 1846, p. 441, pl. xv, figs. 4—6; Thompson, 1856, p. 426.
Ichthyobdella marina, Diesing, 1850, p. 442.
Hirudo vittata, Dalyell, 1853, p. 9, pl. i, figs. 16–21.
(?) *Hirudo campanulata*, Dalyell, 1853, p. 12, pl. i, figs. 26—27.
Pontobdella oligothela, Schmarda, 1861, p. 5, pl. xvi, fig. 144.
(?) *Calliobdella lophii*, van Beneden and Hesse, 1863, p. 36, pl. iii, figs. 11—16.
C. punctata, van Beneden and Hesse, 1863, p. 37, pl. iii, figs. 1—14.
C. striata, van Beneden and Hesse, 1863, p. 38, pl. ii, figs. 1—10.
Pontobdella littoralis, Johnston, 1865, pp. 42 and 304, pl. i, figs. 4—6 (repeated from 1846).
Scorpaenobdella elegans, Saint Loup, 1886, p. 1180.
Calliobdella lubrica, Apáthy, 1888 a, p. 134, *et seq.*, pl. ix, figs. 3 a, b and c, 4 and 9. Apáthy, 1888 c, p. 57.
Calliobdella nigra, Apáthy, 1888 c, p. 58.
Callobdella lubrica, R. Blanchard, 1894 b, p. 14.
Trachelobdella lubrica, R. Blanchard, 1894 b, p. 64.
(?) *Calliobdella lophii*, Johansson, 1898 a, p. 675.

Diagnosis. Body vermiform in young individuals, claviform in adults, blackish yellow or olive, spotted with white. Anterior region with four pairs of lateral non-pulsating tubercles. Clitellum retracted and composed of three large rings followed by six small rings; the male orifice opens between the first and second, and the female orifice between the fourth and fifth, of these small rings.

Each of the first twelve somites of the posterior region of the body bears a pair of lateral pulsating vesicles which, in diastole, arch up the skin of the first two rings. Eight small rings separate the last pair of vesicles from the campanuliform posterior sucker; the anus lies between the antepenultimate ring and the last ring but one. Length 50 mm. in extension; 20—30 mm. in contraction.

[*Note.* The diagnosis of the genus *Trachelobdella* and the description of *T. lubrica* are condensed from those given by R. Blanchard (1894 b). The synonymy of this species is chiefly taken from the same source.]

Hosts. This species is found on the gill-covers, in the pharynx and rarely on the ventral fins of various fish, generally of small size, such as *Scorpaena porcus, Sargus annularis, Corvina umbrina, Caranx trachurus, Uranoscopus scaber, Lophius piscatorius, Blennius pholis, Gobius niger, Coris giofredi, Solea vulgaris* (Apáthy).

Genus: **Piscicola,** *de Blainville,* 1818.

Synonymy:

Piscicola, de Blainville, in Lamarck, 1818. *Haemocharis*, Savigny, 1822, p. 106 (not *Haemocharis*, Filippi, 1837). *Ichthyobdella*, de Blainville, 1827.

Fresh-water leeches, ectoparasitic on fish. Body very long, smooth and cylindrical, the posterior region with paired, lateral, pulsating vesicles. Suckers large and excentrically attached, the posterior and largest with a paramarginal series of oculiform spots. Four eyes upon the anterior sucker, the first pair linear and oblique. Complete somite formed of fourteen rings.

Piscicola geometra, *Linnaeus*, 1761.

Plate XIII, Figs. 4, 5, 6. Text Fig. 2 (p. 135).

Synonymy and Literature:

Hirudo alba perexigua piscibus adhaerens, Aldrovandus, 1602, p. 722.

Hirudo ore caudaque ampla, Frisch, 1729, p. 25, pl. ii; Ledermüller, 1764, pl. lxxxvii, figs. a—i.

Hirudo teres extremitatibus dilatatis, Linnaeus, 1746, p. 365, No. 1275.

Hirudo piscium, Rösel von Rosenhof, 1747, p. 199, pl. xxxii, figs. 1—4; Bergman, 1757, p. 310; O. F. Müller, 1774, p. 43; O. F. Müller, 1776, p. 220; Gmelin, 1788, p. 3097; Bruguière, 1824, p. 133, pl. li, figs. 12—19 (after Rösel); Bosc, 1802, p. 257; Pennant, 1812, p. 70, pl. xxi, fig. 3 (after Rösel); Stewart, 1817, p. 357; Ray Society Reports, 1845, p. 286.

Hirudo dorso elevato, cauda latiore—the great tailed Leech, Hill, 1752, p. 17; Linnaeus, 1758, p. 650.

Hirudo geometra, Linnaeus, 1761, No. 2083; Weser, 1765, p. 44; Linnaeus, 1767, p. 1080, No. 8; Barbut, 1783, p. 20, pl. ii, fig. 7; Pennant, 1778, p. 38, pl. xx, fig. 13; Turton, 1806, p. 70; Turton, 1807, p. 129; Pennant, 1812, p. 70, pl. xxi, fig. 3; Johnson, 1816, p. 35; Brightwell, 1842 a, p. 11, pl. i, figs. 1—8 (coloured); Brightwell, 1842 b, p. 65.

Hirudo galearia, Braun, 1805, p. 35, pl. iii. figs. 1—3 (coloured).

Piscicola piscium, de Blainville, in Lamarck, 1818, p. 294; Milne-Edwards, in Lamarck, 1838, p. 525; Stark, 1828, p. 142; Apáthy, 1888 a, p. 154 *et seq.*, pl. viii, fig. 8 (diagram of somite); Apáthy, 1888 b, p. 774 *et seq.*; Apáthy, 1889, p. 305 (complete somite has 14 rings).

Haemocharis piscium, Savigny, 1822, p. 112.

Piscicola geometra, Moquin-Tandon, 1826, p. 131, pl. vii, fig. 1; Fleming, 1822, p. 604; Leo, 1835, p. 419, pl. xi (anatomy); Henle, 1836, p. 220; Thompson, 1844, p. 437 (occurrence in Ireland); Johnston, 1846, p. 441; Grube, 1851, p. 112; Thompson, 1856, p. 426 (occurrence in Ireland); Johnston, 1865, p. 43; Blanchard, 1894 b, p. 18; Johansson, 1897; Johansson, 1898 a, p. 677; Johansson, 1898 b, p. 581 *et seq.*, fig. 19 (nephridium); Scharff, 1898, p. 190; Plehn, 1898, p. 370; Montgomery, 1899; Brumpt, 1900, p. 47, fig. 1, A—D (cocoon); Castle, 1900, p. 298 (somite growth); Selensky, 1906, p. 33; Johansson, 1909, p. 70, figs. 115—117.

Ichthyobdella geometra, de Blainville, 1827, p. 244; de Blainville, 1828, p. 558, pl. xxxiv, figs. 5, 5 a; Gervais, 1836, p. 628, pl. ccxi, fig. 8; Diesing, 1850, p. 440.

Ichthyobdella percae, Templeton, 1836, p. 236, fig. 28 a, b and c (woodcuts); Diesing, 1850, p. 442.

Ichthyobdella piscium, Egidy, 1844, p. 107, pl. iv, fig. 73.

Piscicola percae, Johnston, 1846, p. 441; Thompson, 1856, p. 426 (occurrence in Ireland); Johnston, 1865, p. 43.

Diagnosis. Body soft and semitransparent, of nearly uniform width posteriorly, attenuated anteriorly, in extension as much as twenty times as long as broad. Anterior sucker circular, about half as wide as the

posterior sucker, the dark pigment upon its upper surface disposed in a more or less distinct cruciform pattern, in the central portion of which are situated the two pairs of eyes. Posterior sucker ovoid, about twice the width of the extended body, with fourteen dark rays and a paramarginal series of fourteen black oculiform spots. Colour greenish, yellowish or brownish, usually finely sprinkled above and below with minute black or brown stellate pigment cells, which are disposed more or less regularly in longitudinal and transverse rows. The body is marked with eight rows of generally elliptical white spots, viz. a pair of marginal rows and, dorsally and ventrally, a single median row situated between a pair of intermediate rows. The spots composing the dorsal median and the two marginal series are the largest and distinguish the first rings (in the middle portion of the body, usually the first four rings) of each somite.

All these spots are subject to considerable variation in form and in the extent to which they are developed. Frequently they tend to fuse into transverse bands in the anterior part of each somite; the three ventral series are the least conspicuous and may be more or less obliterated.

The first eleven somites of the posterior region of the body (XIII—XXIII) are complete with fourteen rings and each of them is provided with a pair of pulsating vesicles which, in diastole, arch up the skin of the first four rings. The male genital orifice lies in somite XI, usually distinguished above by an irregular white blotch, often extending over the whole of its dorsal surface; the female opening lies in somite XII, which under certain conditions may become constricted. Somites I—IV and the anterior portion of V, which is distinguished by a white transverse band, are included in the anterior sucker. Somites VII, VIII and IX are complete. The anus opens in the posterior part of somite XXVI. The seven pairs of rays and oculiform spots seen on the posterior sucker correspond to the seven somites XXVIII—XXXIV of which it is composed. These observations however require revision.

Dimensions. The English living examples which I have examined measured as follows:

Length 20—30 mm.; width 1·5—2 mm.

Larger dimensions appear to be attained. Brightwell's specimens (1842) were "from one to two inches long"; the measurements given by Johansson (1909) are, 20—50 mm. long and 1·2—5 mm. wide.

Distribution, Hosts, etc. *Piscicola geometra* is widely distributed in Europe and not uncommon in the British Islands. It attacks probably most of our species of fresh-water fish and at times is found in considerable numbers by breeders of trout, when these fish are examined during the spawning season. This little leech attaches itself firmly by the posterior sucker to some convenient object, and stretching itself out like a rod and swaying its body to and fro lies in wait for its prey. With the anterior sucker it strikes at and fixes upon any passing fish with remarkable speed and precision and, letting go its hold posteriorly, is carried off attached to its victim. It remains upon its host for some days, drawing blood chiefly from the fins, and drops off when gorged. The process of digestion is comparatively short. The dark brown opaque elliptical cocoons are about 1·5 mm. long and attached to some foreign body.

The rings in this small species cannot be distinguished without the aid of a lens. In preserved specimens they frequently become merged into irregular groups and a correct count is often an impossibility. If not carefully "fixed" the pulsating vesicles usually collapse when the leech dies and in carelessly preserved material they may be inconspicuous or entirely obliterated.

Genus: **Pontobdella,** *Leach,* 1815.

Synonym:

Albione, Savigny, 1822.

Marine leeches, ectoparasitic on skates and rays. Body long and cylindrical, without pulsating vesicles or branchiae, covered by papillae which usually project as conspicuous spiny or warty protuberances but may be partly or entirely retracted. Anterior sucker discoid and excentrically attached, posterior sucker centrally attached and campanulate. Without eyes. Complete somite formed of four rings.

Apáthy (1888 c) has shown that the two species described by Leach (1815) viz. *Pontobdella verrucata* and *P. areolata,* together with the *P. laevis* of de Blainville (1827) merely represent different stages in the appearance of *P. muricata* due to the partial or entire retraction of the warty papillae.

Pontobdella muricata, *Linnaeus*, 1758.

Plate XIII, Figs. 7—12. Text Fig. 3 (p. 147).

Synonymy and Literature:

Hirudo marina, Rondelet, 1554, p. 3; Gesner, 1558, p. 553, fig.; Aldrovandus, 1638, p. 733.

Insectum marinum hirudini affine cornubiense Ray, 1710, p. 4.

Hirudo muricata, Linnaeus, 1754, p. 93, pl. viii, fig. 3; Linnaeus, 1758, p. 650; Linnaeus, 1761, No. 2084; Weser, 1765, p. 44; Linnaeus, 1767, p. 1080; Barbut, 1783, p. 20, pl. ii, fig. 8; Gmelin, 1788, p. 3098; Bosc, 1802, p. 248; Turton, 1806, p. 71; Turton, 1807, p. 130; Pennant, 1812, p. 71, pl. xxi, fig. 4; Pennant, 1766, p. 38, pl. xx, fig. 14; Oken, 1815, p. 371; Johnson, 1816, p. 38; Stewart, 1817, p. 357; Cuvier, 1817, p. 532; Derheims, 1825, pp. 10 and 22; Grant, 1827, No. 14; Dalyell, 1827, p. 391; Dalyell, 1853, p. 3, pl. i, figs. 1—15 (coloured).

Hirudo piscium, Baster, 1760, p. 82, pl. x, fig. 2.

Hirudo blochii, Braun, 1805, p. 43, pl. iv. figs. 1—6.

Hirudo verrucosa, Fleming, 1811, p. 245; Johnson, 1816, p. 39.

Pontobdella spinulosa, Leach, 1815, p. 12, pl. lv, figs. 1, 2; de Blainville, in Lamarck, 1818, p. 294; Risso, 1826, p. 432; de Blainville, 1827, p. 241; de Blainville, 1828, p. 557, pl. xxxiv, figs. 2, 2 a; Stark, 1828, p. 142; Templeton, 1836, p. 236; Grube, 1840, p. 50; Egidy, 1844, p. 106; Johnston, 1846, p. 442; Diesing, 1850, p. 437; Thompson, 1856, p. 427.

Pontobdella verrucata, Leach, 1815, p. 11, pl. lxiv, figs. 1, 2; de Blainville, 1827, p. 242; de Blainville, 1828, p. 557; Milne-Edwards, in Lamarck, 1835, p. 525; Grube, 1840, p. 60; Egidy, 1844, p. 106; Moquin-Tandon, 1846, p. 288, pl. ii, figs. 10, 11 (coloured); Diesing, 1850, p. 438; Grube, 1851, p. 108.

Pontobdella areolata, Leach, 1815, p. 10, pl. lxiii; de Blainville, 1827, p. 242; de Blainville, 1828, p. 557; Moquin-Tandon, 1846, p. 290, pl. ii, fig. 12 (coloured); Diesing, 1850, p. 439; Grube, 1851, p. 108.

Pontobdella muricata, de Blainville, in Lamarck, 1818, p. 293; Risso, 1826, p. 432; de Blainville, 1827, p. 242; de Blainville, 1828, p. 557; Stark, 1828, p. 142; Templeton, 1836, p. 236; Egidy, 1844, p. 106, pl. iv. fig. 71; Grube, 1840, p. 60; Moquin-Tandon, 1846, p. 285, pl. i, figs. 11 (coloured) and 12, and pl. ii, figs. 1—9 (anatomy); Grube, 1851, p. 108; Thompson, 1856, p. 426 (in Ireland); van Beneden and Hesse, 1863, p. 23, pl. i, figs. 1—6; Vaillant, 1870; M'Intosh, 1874, p. 192; M'Intosh, 1875, p. 114, pl. v, fig. 1 (coloured); Bourne, 1884; Dutilleul, 1885, p. 349 and 1886a, p. 127, pl. i (genital organs); Dutilleul, 1886b, p. 559, and 1886c, p. 572, pl. xii (anatomy); Apáthy, 1888a, p. 153, etc., pl. viii, figs. 5, 16 and 17 (head) and fig. 6 (diagram of somite); Apáthy, 1888c, p. 47 *et seq.*; Rhode, 1891 (nervous system); Gibbs, 1898, p. 330 (habits); Johansson, 1898a, p. 668; Johansson, 1898b, pp. 582 *et seq.* (anatomy); Robertson, 1909, p. 119, pl. ix (*Trypanosoma raiae* in alimentary canal).

Albione muricata, Savigny, 1822, p. 110; Delle Chiaje, 1823, p. 49, pl. i, fig. 14; Moquin-Tandon, 1826, p. 136, pl. vii, fig. 4.

Albione verrucata, Savigny, 1822, p. 111 ; Moquin-Tandon, 1826, p. 137, pl. vii, fig. 5.
Sipunculus marinus, De Serres, 1822, p. 61.
Sanguisuga muricata, Bruguière, 1824, p. 133, pl. lii, fig. 5.
Albione areolata, Moquin-Tandon, 1826, p. 138.
Pontobdella laevis, de Blainville, 1827, p. 243 ; de Blainville, 1828, p. 557, pl. xxxiv, fig. 3 ; Moquin-Tandon, 1846, p. 290 ; Thompson, 1846, p. 391 (recorded from Ireland) ; Diesing, 1850, p. 439 ; Grube, 1851, p. 108 ; Thompson, 1856, p. 427.
Pontobdella verrucosa, Leydig, 1851, p. 318, pl. ix, fig. 2 (anatomy).

Diagnosis. Body cylindrical, fusiform, much attenuated anteriorly, grayish green or brownish green, somewhat lighter on the ventral surface, with irregular dark brown spots. Anterior sucker with six small marginal papillae.

The anterior sucker includes the first four somites. Somites V, VI, X, XI, XII and XXIV—XXVII biannulate, VII, VIII and IX triannulate ; the eleven somites XIII—XXIII following the clitellum, are complete with four rings. The first ring of the complete somite is the largest, the third is the smallest, the second and fourth are of equal length. The papillae in each somite are disposed upon their several rings according to a definite and characteristic pattern which however may become modified to a certain extent when, as not infrequently happens, some papillae are missing or extra ones are intercalated. The typical arrangement on the dorsal surface is indicated in Text Fig. 3 (p. 147). The largest papillae occur on the first ring of the somite and are eight in number, and there are usually ten upon the second and twelve upon the fourth ring.

The papillae may be prominent, conical and terminated by an array of spiny tubercles forming a kind of rosette [the typical form], less acute, mammiform and without the terminal rosettes [*P. verrucata*], so far sunk into the body as to leave an irregular basal marking [*P. areolata*] or entirely retracted, leaving the surface smooth [*P. laevis*].

The clitellum extends from the second and last ring of somite X up to and including the last ring of somite XII.

The male genital orifice lies between the two rings of somite XI, that is, between the 16th and 17th rings following the anterior sucker. The female orifice is situated two rings behind the male, between the first and second rings of somite XII.

The anus lies upon the first ring or between the two rings of somite XXVI. The crop has a single undivided caecum reflected posteriorly.

Length, at rest, 75—100 mm. ; fully extended, up to 200 mm. Width 8—15 mm.

Distribution, Hosts, etc. *Pontobdella muricata* is parasitic on *Raia batis* and other species of skate and has been recorded also from *Torpedo marmorata*. It is found in the Mediterranean, and on the western and northern coasts of Europe, and is of fairly frequent occurrence in British waters, where it is known to fishermen as the "skate leech" or "skate sucker." The egg capsules are opaque, tough, leathery, barrel-shaped structures about 5 mm. long and 4 mm. in width, attached by a pedicle to foreign bodies. One leech will produce a considerable number of these capsules, one after another, at short intervals ; the interior of some empty bivalve shell appears to be a favourite place for their deposition and they are found in groups containing from three or four to fifty or more ; fifty-four is the largest number as yet observed in a group (Dalyell). The process of digestion in this species, as in *Hirudo medicinalis*, is exceptionally slow, and when fully gorged it can live for many months without taking food. It has been stated that *P. muricata* is unable to swim ; when not too fully distended with blood it can and does swim, the body being somewhat flattened for the purpose, after the manner of other leeches. It is usually however a very sluggish animal, remaining for long periods attached to some convenient object by its powerful posterior sucker, the body tightly curled upon itself or more or less extended and unrolled.

Several attempts, all of them unsatisfactory, have been made to account for the existence of the warts which form such a characteristic feature of this species. In this connection we may note the striking resemblance both in colour and in form between this leech and the thorny body of its host.

Family II. **GLOSSOSIPHONIDAE.**

Synonym :
Clepsinidae.

Fresh-water Rhynchobdellae with ovate, flattened, never cylindrical body. Differentiation of the head region into a permanent anterior sucker distinct from the body may occur, but never to the same extent as in the Ichthyobdellidae. Crop and stomach with conspicuous, paired lateral caeca ; the stomach always with four pairs. The eggs are usually fixed, and the young attach themselves to the ventral surface of the parent. Certain species deposit their eggs upon foreign bodies and brood over them.

Genus: **Protoclepsis**, *Livanow*, 1902.

Synonymy:

Glossiphonia, Johnson, 1816 (partim). *Clepsine*, Savigny, 1822 (partim). *Haemocharis*, de Filippi, 1837 (partim ; not *Haemocharis*, Savigny, 1822). *Theromyzon*, Philippi, 1867. *Hemiclepsis*, Vejdovsky, 1883 (partim). (?) *Protoclepsine*, Moore, 1898.

Glossosiphonidae of medium size, with four pairs of eyes. Complete somite formed of three rings. Somites III—XXIII are complete. Crop with more than six pairs of many-lobed lateral caeca, the last and longest pair reflected posteriorly. "The presence of two nuclei in each muscle cell is a special peculiarity." (Livanow.)

This genus has been created by Livanow (1903, p. 339) in order to admit *P. tessellata*, a species formerly included in *Hemiclepsis* (*q.v.*) and several eight-eyed forms occurring for the most part in Lake Baikal. These comprise a well-defined and homogeneous little group, the members of which are somewhat difficult to distinguish one from another. This close similarity, if we are to believe Livanow, has led to the confusion of two species under the name *Hirudo tessellata* (O. F. Müller 1774.) Such a possibility had already been contemplated by Blanchard (1892, p. 62.) The species which Livanow has isolated from the *H. tessellata* of previous authors has been described by him under the name of *Protoclepsis meyeri*. The genus *Protoclepsis* is divided by its author into two groups characterized as follows:

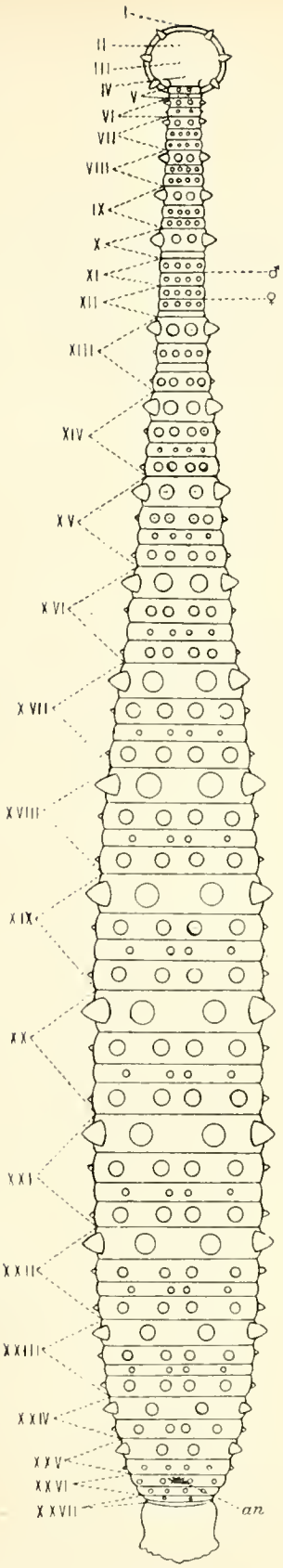
A. Genital apertures separated by two rings. A primitive form of vagina is present in adults. It is undeveloped in young individuals and the oviducts opening directly to the exterior on either side of the ventral median line appear as two separate female genital apertures.

B. Genital apertures separated by more than two rings. The female opening is the single aperture of a well-developed vagina.

P. meyeri falls within the first and *P. tessellata* within the second of these groups.

The synonymy of *P. tessellata* and *P. meyeri* is inextricably confused, since the inadequacy of the descriptions given by most writers renders it impossible to determine which of these two species they had in view.

Only Malm and R. Blanchard have noted the position of the genital orifices; to the latter we owe the first satisfactory diagnosis of *P. tessellata*, whilst in the description of Malm (1860) we have the only *positive* record of *P. meyeri* in Western Europe.



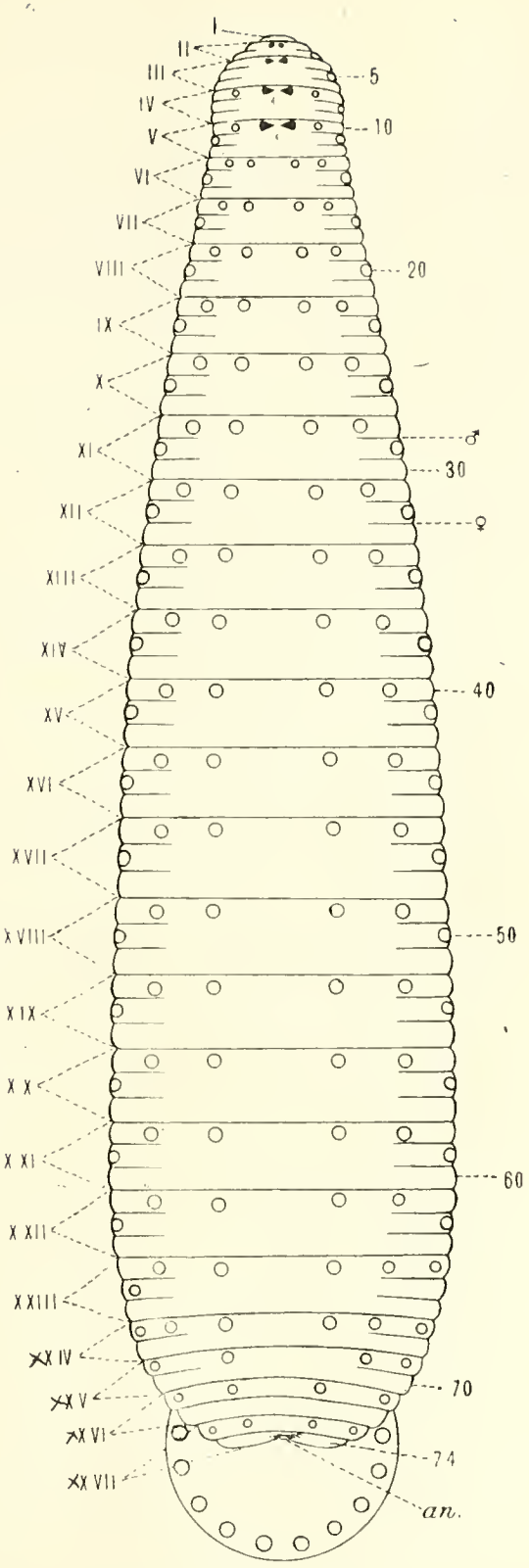


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Fig. 3. *Pontobdella muricata.*

Fig. 4. *Protoclepsis tessellata.*

Fig. 3. *Pontobdella muricata.* Diagram showing annulation and external features on dorsal surface. Somites indicated in Roman numerals.

Fig. 4. *Protoclepsis tessellata.* Diagram showing annulation, position of spots and other external features on dorsal surface. *an.* Anus. Somites indicated in Roman numerals and rings in figures.

That *P. tessellata* occurs in England is proved by the discovery of the example which forms the subject of the accompanying description and figures, and this fact substantiates the opinion expressed by Livanow and shared by the present writer, that Brightwell (*Nephelis tessellata*, 1842), Thompson (*Glossiphonia eacheana*, 1856) and Houghton (*G. tessellata*, 1865) all had this form, and not *P. meyeri*, before them.

Protoclepsis tessellata, *O. F. Müller*, 1774.

Plate XV, Figs. 33—35. Text Fig. 4 (p. 147).

Synonymy and Literature:

Hirudo tessulata, O. F. Müller, 1774, p. 45 ; O. F. Müller, 1776, p. 220 ; Gmelin, 1788, p. 3098; Schrank, 1803, p. 161 ; Braun, 1805, p. 56, pl. vi, figs. 6—10 (coloured); Turton, 1806, p. 70 ; Johnson, 1816, p. 33 ; Fleming, 1824, p. 400.

Hirudo tessellata, Bosc, 1802, p. 247 ; de Blainville, 1827, p. 261 ; Dalyell, 1853, p. 38, pl. iv, figs. 24—30 (coloured).

Erpobdella tessulata, Fleming, 1822, p. 604 ; Thompson, 1844, p. 437 (in Ireland); Johnston, 1846, p. 440.

Ichthyobdella tessellata, de Blainville, 1828, p. 558.

Erpobdella vulgaris, var. tessellata, de Blainville, 1828, p. 564.

Nephelis tesselata, Savigny, 1822; Brightwell, 1842, p. 13, pl. i, figs. 15—17 (coloured).

Clepsine tessulata, Fr. Müller, 1844 a, p. 376 ; Fr. Müller, 1844 b, p. 21 ; Fr. Müller, 1846, p. 138, pl. viii, figs. 1—4 and 7—13 (anatomy of genital organs); Diesing, 1850, p. 447 ; Diesing, 1858, p. 495 ; Grube, 1851, p. 114 ; Malm, 1860, p. 81 ; Örley, 1886, p. 100 ; Apáthy, 1888 a, p. 154 *et seq.*, pl. viii, fig. 14 (head); Apáthy, 1888 b, p. 789 *et seq.*; Spoof, 1889, p. 16 ; Brandes, 1900, p. 126 (copulation).

Glossiphonia tessellata, Moquin-Tandon, 1846, p. 379 ; Thompson, 1846, p. 390 (in Ireland); Johnston, 1865, p. 50 ; Houghton, 1865, p. 88 *et seq.* ; R. Blanchard, 1892 f, p. 56 (description); R. Blanchard, 1892 g, p. 117 (in Chile); de Guerne, 1892, p. 360 (on waterfowl); R. Blanchard, 1893 b, p. 23 (in Piedmont); R. Blanchard, 1893 h, p. 197 ; R. Blanchard, 1893 i, p. 2 (in Syria) ; Mégnin, 1905, p. 71 (on waterfowl).

Hirudo vitrina—the Glassy Leech, Dalyell, 1853, p. 42, pl. v, figs. 20—23 (coloured); Johnston, 1865, p. 53.

Glossiphonia eacheana, Thompson, 1846, p. 390 ; Thompson, 1856, p. 425 ; Johnston, 1865, p. 54.

Haemocharis eacheana, Thompson, 1856 b, p. 425.

Hemiclepsis tessellata, Vejdovsky, 1883 ; R. Blanchard, 1894 b, p. 32 ; Bayer, 1898, p. 648 *et seq.* (sense organs); Scharff, 1898, p. 192 (in Ireland).

Clepsine tesselata, Weltner, 1887, p. 85 ; Collin, 1892, p. 164 (from *Cygnus atratus*).

Diagnosis. Body flattened and very soft; in extension ovate-oblong, with nearly parallel sides and the head region slightly dilated; in extreme contraction ovoid, the length being equal to about twice the

breadth. Colour grayish olive above, pale gray beneath; the anterior extremity and the posterior sucker are finely sprinkled with black, stellate, superficial pigment cells.

Dorsal surface with six longitudinal series of rounded yellowish spots. The spots composing four of these series occur on the first ring of each somite and correspond to the outer paramedian and intermediate sense organs, the inner paramarginal sense organs on this ring being without spots, whilst the spots composing the two remaining series are situated on the outer paramarginal lines on the second ring of each somite. The paramarginal spots are the largest and sometimes overlap on to the ventral surface. All the spots are subject to individual fluctuations in size; usually some of them are imperfectly developed or absent. Papillae, generally small and scarcely perceptible, occur dorsally on the first rings of each somite and correspond to the outer paramedian, intermediate and inner paramarginal sense organs. Similar papillae are situated on the outer paramedian and inner paramarginal lines and occasionally (Livanow) on the intermediate lines on the first rings of each somite on the ventral surface. These are not associated with yellow spots.

74 rings. Somite I uniannulate, somites II and XXIV—XXVII biannulate; the 21 somites III—XXIII are complete with three rings.

The four pairs of eyes lie respectively on the first rings of somites II, III, IV and V, that is, upon rings 2, 4, 7 and 10.

The male genital orifice lies between rings 28 and 29, that is, between the first and second rings of somite XI; the female orifice is four rings behind the male, between rings 32 and 33.

The anus opens behind ring 73 and is separated by the seventy-fourth and last ring, which is incomplete, from the posterior sucker.

Dimensions. The following measurements are taken from the single living English example which I have had the opportunity of examining.

At rest, but moderately extended, 16 mm. long and 2 mm. wide. At rest, in extreme contraction, about 8 mm. long and 4 mm. wide. In extreme extension, 24 mm. long.

Larger dimensions are probably attained; the results of the following observers are however somewhat conflicting:—O. F. Müller, 1774, 18 lines long and 5 lines wide; Brightwell, 1842 (English example), "about an inch long"; Moquin-Tandon, 1846, 18—25 mm. long and 4—5 mm. wide; Thompson, 1846 (Irish examples) "size commonly...nine lines";

Houghton, 1865 (English examples) "nearly an inch in length"; Johnston, 1865 (English examples), 18 lines long and 5 lines wide; Dalyell, 1853 (Scotch examples), at rest, 5—6 lines long and about 2½ inches long when extended; Blanchard, 1893 h, 24 mm. long and 10 mm. wide (in alcohol); Livanow, 1902, 15 mm. long and 3 mm. wide; Johansson, 1909, 10—30 mm. long and 2—6 mm. wide.

Distribution, Habits. *P. tessellata* is an extraordinarily active and restless animal, starting into movement upon the slightest disturbance. It is very prolific, carrying, according to Houghton, as many as 200 young, and more than 300, if we are to believe O. F. Müller.

Its range appears to be confined to Europe and, perhaps, adjacent parts of Asia; the single example recorded by Blanchard (1892 g) from Chile (on the body of a rat, *Myopotamus coypu*) may probably have been introduced, as that writer believes, by artificial means. It is nowhere abundant and is of rare occurrence in the British Islands. Fleming (1822) first included it among the British species. Brightwell (1842) described a single example from the River Wensum, at Costessy, near Norwich, and Houghton (1865, p. 88), who considered it to be less rare than generally supposed, obtained it in weedy pools and found it "not uncommon in the Shropshire Union Canal." Johnston (1865) catalogues a specimen from Holy Island Lough, and recently, I have taken from a stagnant, weedy pond at Histon, near Cambridge, a single individual of which coloured figures accompany this description.

In Scotland it is "rarely disseminated" according to Dalyell (1853), who records it from Coldingham Loch, Berwickshire, the counties of Edinburgh and Linlithgow and the Island of Bute.

Scharff (1898) states that it is rare in Ireland and in addition to Tuam and Lough Neagh, where it was found by Thompson (1844), records it from Clonbrock, from Santry, Co. Dublin, and from Glenomeragh, Co. Clare.

Hosts. There appears to be no doubt that *P. tessellata* is parasitic upon various species of waterfowl and particularly upon the fresh-water ducks (*Anatinae*) although, in the instances of parasitism cited below, we cannot state with certainty in every case whether the descriptions apply to this leech or to some closely allied species such as *P. meyeri*. Weltner (1887, p. 85) states that at a farm at Wanzenau, near Strasbourg, the ducks and geese were nearly all destroyed by a leech, described as *Glossiphonia* (*Protoclepsis*) *tessellata*, which attached itself to the walls of the oesophagus.

De Guerne (1892) has collected interesting evidence showing that migrating ducks can become active agents in the distribution of living leeches attached to their bodies. Leeches obtained by him from the breast plumage of a wigeon (*Mareca penelope* L.) and a teal (*Querquedula crecca* L.) were described by Blanchard (1893, p. 62) as *P. tessellata*, though Livanow would have us believe that they were specimens of *P. meyeri*.

Blanchard (1893 h, p. 197) describes two undoubted examples of *P. tessellata*, taken from the nasal cavities of *Anas glacialis*.

Finally Mégnin (1905, p. 71) instances the case of a domestic duck choked by an accumulation of leeches, also referred by Blanchard to *P. tessellata*, which had penetrated into the air passages and completely blocked the trachea.

Genus: **Hemiclepsis**, *Vejdovsky*, 1883.

Synonymy:

Haemocharis, de Filippi, 1837 (partim; not *Haemocharis*, Savigny, 1822). *Glossiphonia*, Johnson, 1816 (partim). *Clepsine*, Savigny, 1822 (partim). *Hemiclepsis*, Vejdovsky, 1883 (partim).

Glossosiphonidae of medium size, with two pairs of eyes. Complete somite formed of three rings. The 21 *somites III—XXIII are complete. Head region dilated into a permanent anterior sucker distinct from the body. Crop with more than six pairs of many-lobed lateral caeca, the last and longest pair reflected posteriorly.*

This genus has recently been revised by Livanow (1902) who has separated from it the genus *Protoclepsis* (*q.v.*).

Hemiclepsis marginata, *O. F. Müller*, 1774.

Plate XIV, Figs. 28—32. Text Figs. 5 and 6 (p. 153).

Synonymy and Literature:

Hirudo marginata, O. F. Müller, 1774, p. 46; O. F. Müller, 1776, p. 200; Gmelin, 1788, p. 3098; Bosc, 1802, p. 257; Schrank, 1803, p. 162; Turton, 1806, p. 70; Johnson, 1816, p. 36; Baer, 1827, p. 728, pl. xxxii, fig. 10.

Hirudo variegata, Braun, 1805, p. 61, pl. vii, figs. 1—6 (coloured).

Hirudo cephalota, Carena, 1820, p. 298, pl. xii, figs. 19, 20; Carena, 1823, p. 336; de Blainville, 1827, p. 266, pl. xxvii, figs. 5, 5 a (named in plate *Ichthyobdella cephalota*).

Hirudo oscillatoria, Saint Amans, 1825, p. 193, pl. viii.

Piscicola marginata, Moquin-Tandon, 1826, p. 133, pl. vii, fig. 2.
Piscicola tesselata, Moquin-Tandon, 1826, p. 133.
Glossobdella cephalota, de Blainville, 1827, p. 266.
Ichthyobdella marginata, de Blainville, 1828, p. 558.
Haemocharis marginata, de Filippi, 1837, p. 26.
Clepsine marginata, Fr. Müller, 1844, p. 377, pl. x, fig. 4; Diesing, 1850, p. 447; Apáthy, 1888 a, p. 154, etc., pl. ix, fig. 1 (head); Apáthy, 1888 b, p. 789, etc.; Whitman, 1878, p. 2 *et seq.* (development); Leuckart, 1894, fig. 280 (digestive tract); Oka, 1894, p. 81 *et seq.*, pl. v, fig. 24, etc. (sinus system, nephridia).
Glossiphonia marginata, Moquin-Tandon, 1846, p. 375, pl. xiv, figs. 10—20; Houghton, 1860, p. 248, pl. xvi, C, figs. 1, 2 (first record in England); Houghton, 1861, p. 34, pl. iii, fig. 15 (coloured); Houghton, 1865, p. 83, pl. i, fig. 10 (coloured); R. Blanchard, 1892 b, p. 173 (description).
Hirudo flava—the Yellow Leech, Dalyell, 1853, p. 45, pl. v, figs. 1—19 (coloured).
Glossiphonia flava, Johnston, 1865, p. 53.

Diagnosis. Body claviform, flattened and more or less transparent. With the exception of the margins and anterior extremity, which are colourless or hyaline, the ground colour is pale yellow, variegated above with orange, reddish brown, lemon yellow and an intense green. Usually the green pigment predominates; there is however a form in which it is entirely absent, leaving the body yellow. The general colouration is considerably modified when the crop is distended with blood. Green or reddish brown transverse stripes traverse the clear margins of the first rings of each somite.

Dorsal surface with seven longitudinal series of lemon yellow spots. The spots composing four of these rows occur on the first rings of each somite and correspond to the outer paramedian and intermediate sense organs.

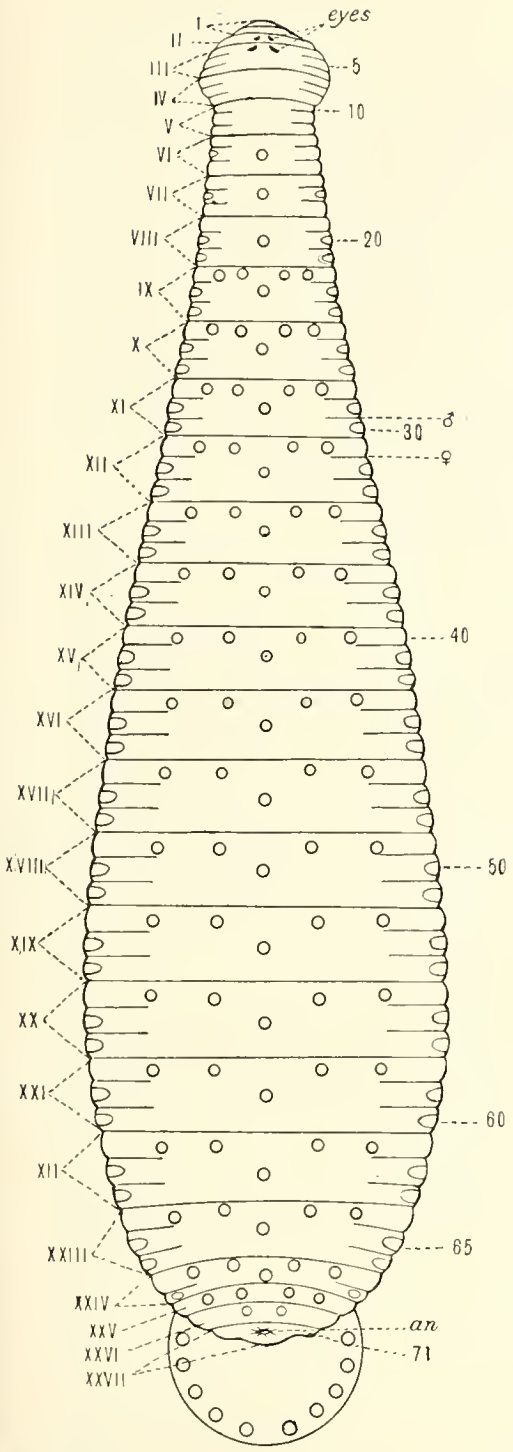
The spots composing a median row fall upon the second rings of each somite, whilst those forming the two remaining series occupy a paramarginal position and occur on both the second and third rings of each somite

These spots are subject to individual fluctuations in size and may not all be present. The right and left paramarginal spots in each somite not infrequently are fused together, forming irregular **C**-shaped markings.

Low papillae correspond to the outer paramedian and intermediate sense organs and spots on the first ring of each somite.

Ventral surface without spots or papillae.

Posterior sucker with a paramarginal row of lemon yellow spots, between which reddish brown rays frequently appear.



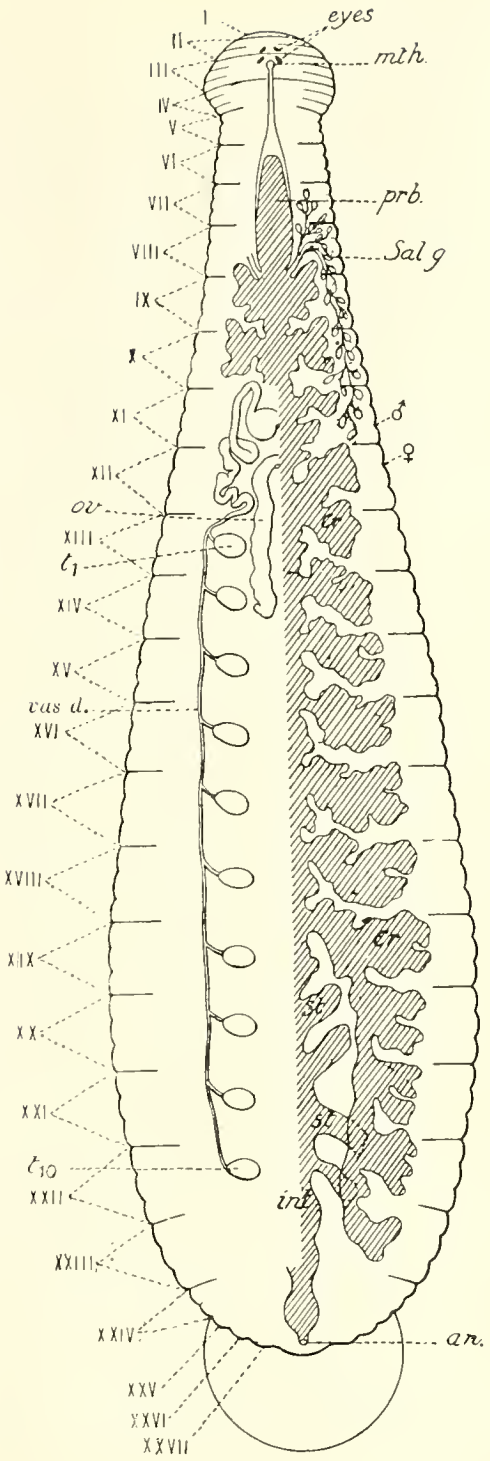


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Fig. 5. *Hemiclepsis marginata.*

Fig. 6. *Hemiclepsis marginata.*

Fig. 5. *Hemiclepsis marginata.* Diagram showing annulation, position of spots, etc., on dorsal surface. Somites in Roman numerals, rings in figures.

Fig. 6. The same. Alimentary tract shown on the right side; reproductive system on the left. *mth.* Mouth. *prb.* Proboscis. *Sal. g.* Salivary glands. *Cr.* Crop. *st.* Stomach. *int.* Intestine. *ov.* Ovary. *vas d.* Vas deferens. *t* 1, *t* 10, Testes of the first and tenth pairs. *an.* Anus. (Schematic.)

72 rings, of which the last is inconspicuous and incomplete. Somites I, XXIV and XXVII biannulate; II, XXV and XXVI uniannulate, the 21 somites III—XXIII complete with three rings.

The first pair of eyes lies on ring 3; the second and largest pair is situated on ring 4, that is, upon the first ring of somite IV.

The proboscis is short, extending posteriorly to the last ring of somite VIII. The mouth opens in the posterior part of somite III.

The male genital orifice lies between rings 29 and 30, that is, between the second and third rings of somite XI; the female opening is two rings behind the male, between rings 31 and 32. Testes 10 pairs.

The anus lies behind the first ring of somite XXVII.

Eggs attached to the ventral surface of the parent.

Length, at rest, 16—18 mm., width, at rest, 2·5—8 mm. Length, in extreme extension, 27—30 mm.

Distribution, Food, etc. This beautiful species occurs throughout the greater part of Europe. It has not been recorded from Ireland. It is rare in Scotland, according to Dalyell (1853, pp. 45—49, pl. v, figs. 1—19), who describes the yellow form already referred to, under the name *Hirudo flava*. It is equally rare in England, where it was first recorded in 1859 by Houghton (1860, p. 248, pl. xvi, C, figs. 1—2, who took one example in Bala Lake and several others in a small stream near Solihull in Warwickshire, and again later (1865, p. 89) "on one or two occasions...found it in the Shropshire Union Canal."

Since 1865 it does not appear to have been noticed in Great Britain, probably because it has not been sought for. Recently (1898—9) I have taken a number of examples from a stagnant weedy pond, stocked with fish, at Histon, near Cambridge. These were found among aquatic plants together with other *Glossosiphonidae*.

This species is a fish parasite (Houghton, 1865, p. 89; Whitman, 1878, p. 10; Blanchard, 1884, p. 33). Apáthy (1888 b) states that it attacks the smaller species of carp.

Genus: **Glossosiphonia**, *Johnson*, 1816.

Synonymy:

Glossiphonia, Johnson, 1816. *Glossopora*, Johnson, 1817. *Clepsine*, Savigny, 1822. *Erpobdella*, de Blainville, in Lamarck, 1818. *Glossobdella*, de Blainville, 1827. *Clepsina*, de Filippi, 1837. *Glossosiphonia*, R. Blanchard, 1894.

Glossosiphonidae of small or medium size, with three pairs of eyes. Complete somite formed of three rings. Crop with six pairs of sub-lobate lateral caeca, the last and longest pair reflected posteriorly.

Glossosiphonia heteroclita, *Linnaeus*, 1761.

Plate XIV, Figs. 18—21. Text Fig. 7 (p. 157).

Synonymy and Literature:

Un Ver plat et blanc, Trembley, 1744, p. 147, pl. vii, fig. 7; Ledermüller, 1764, p. 165, pl. lxxxiv, figs. k—q.

Hirudo heteroclita, Linnaeus, 1761, No. 2085; Weser, 1765, p. 44; Linnaeus, 1767, p. 1080; Turton, 1806, p. 70; Johnson, 1816, p. 34.

Hirudo hyalina, O. F. Müller, 1774, p. 49; O. F. Müller, 1776, p. 220; Gmelin, 1788, p. 3097; Bosc, 1802, p. 256; Schrank, 1803, p. 163; Stewart, 1817, p. 357; Baer, 1827, p. 728; pl. xxxii, fig. 11.

Hirudo papillosa, Braun, 1805, p. 64, pl. vii, figs. 7—10 (coloured).

Hirudo trioculata, Carena, 1820, p. 303, pl. xii, fig. 22; Carena, 1823, p. 334.

Clepsine hyalina, Moquin-Tandon, 1826, p. 106; Müller, F., 1844 b, p. 27; Diesing, 1850, p. 453.

Clepsine carenae, Moquin-Tandon, 1826, p. 105, pl. iv, fig. 4; Diesing, 1850, p. 454.

Glossobdella hyalina, de Blainville, 1827, p. 263; de Blainville, 1828, p. 565.

Glossobdella trioculata, de Blainville, 1827, p. 267, pl. xxxvii, fig. 4.

Glossobdella carenae, de Blainville, 1828, p. 565.

Clepsina carenae, de Filippi, 1839, p. 6.

Clepsina hyalina, Brightwell, 1842, p. 15, pl. i, figs. 18 and 19 (erroneously named *C. complanata*; fig. 20 represents, not *C. hyalina*, but *C. complanata*); Thompson, 1844, p. 437 (recorded from Ireland).

Glossopora (?) *hyalina*, Johnston, 1846, p. 440.

Glossiphonia hetcroclita, Moquin-Tandon, 1846, p. 358, pl. xiii, figs. 1—6 (coloured); Johnston, 1865, p. 52; Castle, 1900 a, p. 42, pl. v and pl. viii, figs. 35—38 (complete description).

Glossiphonia carenae, Moquin-Tandon, 1846, p. 362, pl. xiii, figs. 7—9 (coloured).

Clepsine papillosa, Grube, 1851, p. 113.

Glossiphonia hyalina, Thompson, 1856, p. 425 (occurrence in Ireland).

Glossiphonia hyalina, Houghton, 1861, p. 33 *et seq.*; Houghton, 1865, p. 82, pl. i, figs. 5 and 6 (coloured).

Clepsine heteroclita, Whitman, 1878, p. 2 *et seq.* (development); Orley, 1886; Apáthy, 1888 a, p. 154, pl. vi, fig. 3 (head region); Oka, 1894, p. 81 *et seq.* (sinus system, nephridia); Leuckart, 1894, p. 552, fig. 237 (head) and fig. 239 (sense organ); Scharff, 1898, p. 191 (occurrence in Ireland).

Glossiphonia trioculata, R. Blanchard, 1893 b, p. 4.

Glossosiphonia heteroclita, R. Blanchard, 1894 b, p. 26 (diagnosis); Johansson, 1909, p. 75, figs. 127, 128.

Diagnosis. Body ovate-acuminate, much flattened, without papillae, of a clear and pellucid amber yellow colour. Minute brownish or blackish spots, which tend to group themselves into transverse striae upon the third ring of each somite, may be present on the dorsal surface.

The three pairs of eyes are somewhat variable in position. The eyes forming the anterior and smallest pair are closely approximated (in an inner paramedian position) and lie, generally in ring 5, occasionally in ring 4 or in ring 6 ; one or both of them may be devoid of pigment. The eyes composing the second and third pairs are wider apart (in an outer paramedian position) and situated respectively in rings 7 and 8.

71 rings. The male and female genital ducts have a common orifice between rings 28 and 29. Testes, 6 pairs. The anus lies between rings 70 and 71.

Eggs attached to the ventral surface of the parent.

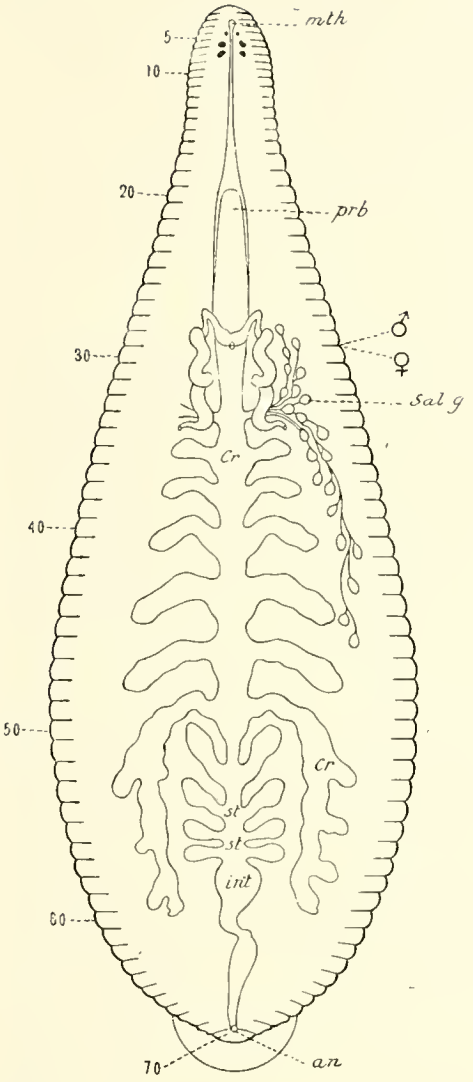
Length, at rest, 10—13 mm. ; width, at rest, 3—4·5 mm.

Distribution, Food, etc. A sluggish species, common in England, in stagnant and slowly running waters, chiefly among aquatic plants. Its range probably extends into Scotland but its occurrence there does not seem to have been recorded. In Ireland it is found in a few localities but is not common (Scharff, 1898). It is widely distributed in central Europe and occurs in Sweden, in Sardinia (Blanchard, 1894) and in North America (Castle, 1900).

It breeds in England in June and July: large individuals may carry more than 60 eggs. It is parasitic chiefly upon gasteropods.

Varieties. Apáthy (1888 b, p. 790) states that the colouration of this leech shows "numerous transitions to a variety (*striata*) which is distinguished by intensely black transverse stripes, more or less interrupted, on every third ring." Castle (1900, p. 42, pl. viii, fig. 38) finds in the United States all gradations between the clear yellow form and a form with transverse striae and an irregular longitudinal band on the dorsal surface, due to the presence of orange, dark brown or black superficial pigment cells. Dark brown or blackish pigment is rarely present in British examples of *G. heteroclita*. Houghton (1865) does not refer to it and among a very large number of individuals examined, I have not found one in which it occurred. Johnston however states in his *Catalogue* of British species (1865) that "the back is sometimes speckled with blackish dots."

In rare cases not only the eyes composing the first pair but also the right and left components of the second and third pairs are so closely approximated as to give the appearance of three single eyes. On this trioculate and triangular disposition Carena (1820) founded his supposed species *Hirudo trioculata*.



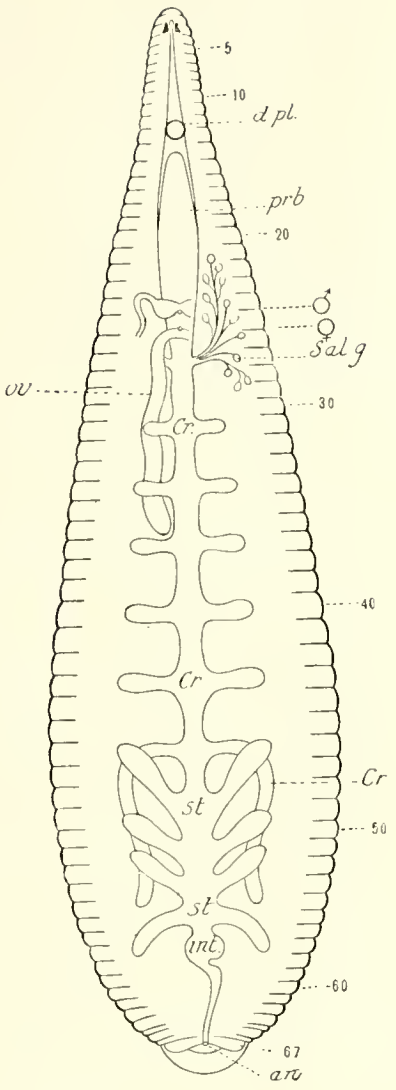


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Fig. 7. *Glossosiphonia heteroclita.*

Fig. 8. *Helobdella stagnalis.*

Fig. 7. *Glossosiphonia heteroclita.* Annulation and digestive tract. *mth.* Mouth. *prb.* Proboscis. *sal. g.* Salivary glands. *Cr.* Crop. *st.* Stomach. *int.* Intestine. *an.* Anus. Ovary omitted. (Schematic.)

Fig. 8. *Helobdella stagnalis.* Annulation, digestive tract, etc. *d. pl.* Dorsal plate. Other lettering as before. (Schematic.)

Among a number of individuals taken from the same locality one often may find forms exhibiting stages intermediate between the normal and the trioculate type and not infrequently examples in which the eyes are more or less asymmetrically disposed.

Glossosiphonia complanata, *Linnaeus*, 1758.

Plate XIV, Figs. 22—27. Text Figs. 9 and 10 (p. 161).

Synonymy and Literature:

A very small sort of Leech, (?) Baker, 1753, p. 415.

Hirudo lateribus attenuatis—the Snail-leech, Hill, 1752, p. 16.

Hirudo sexoculata, Bergman, 1757, p. 313, pl. vi, figs. 12, 13; Schrank, 1803, p. 162.

Hirudo complanata, Linnaeus, 1758, p. 650; Linnaeus, 1761, No. 2082; Weser, 1765, p. 44; Linnaeus, 1767, p. 1079; O. F. Müller, 1774, p. 47; O. F. Müller, 1776, p. 220; Gmelin, 1788, p. 3097; Ure, 1793, p. 233; Bosc, 1802, p. 256; Braun, 1805, p. 58, pl. vi, figs. 11—16 (coloured); Turton, 1806, p. 69; Turton, 1807, p. 129; Pennant, 1812, p. 72; Stewart, 1817, p. 357; Carena, 1820, p. 97, pl. xii, figs. 17, 18; Derheims, 1825, p. 10; Dalyell, 1853, p. 30, pl. iv, figs. 1—16 (coloured).

Hirudo crenata, Kirby, 1795, p. 320, pl. xxix; Turton, 1806, p. 71; Turton, 1807, p. 129.

Hirudo crinata, Pennant, 1812, p. 72.

Glossiphonia tuberculata, Johnson, 1816, p. 25.

Glossopora tuberculata, Johnson, 1817, p. 346, pl. xvii, figs. 1—10; Johnson, 1825, p. 49, pl. xvii, figs. 1—10; Stark, 1828, p. 142; Thompson, p. 482.

Erpobdella complanata, de Blainville (in Lamarck), 1818, p. 296; de Blainville, 1827, p. 263; Templeton, 1836, p. 235.

Clepsine complanata, Savigny, 1822, p. 120; Savigny, 1826, p. 463; Moquin-Tandon, 1826, p. 101, pl. iv, fig. 1; Risso, 1826, p. 431; Fr. Müller, 1844 b, p. 25; E. Blanchard, 1845, p. 377, pl. xviii, fig. 9 (nervous system); Leydig, 1849, p. 2; pl. iii, figs. 1—11 (anatomy); Diesing, 1850, p. 452; Grube, 1851, p. 113; Picaglia, 1877, p. 153; Whitman, 1878, p. 2 *et seq.* (development); Schultze, 1883, p. 80, figs. 1—4 (nephridia); Nussbaum, 1885, p. 181 (development of reproductive organs); Oka, 1894, p. 81 *et seq.*, pl. iv, figs. 4—15, pl. v, fig. 16, etc., pl. vi, fig. 39, etc. (sinus system, nephridia); Leuckart, p. 584, fig. 297; R. Blanchard, 1896 c, p. 140 (in North America).

Glossopora complanata, Fleming, 1822, p. 604; Johnston, 1846, p. 440.

Sanguisuga complanata, Bruguière, 1824, p. 132, pl. li, figs. 20, 21 and A.

Glossobdella complanata, de Blainville, 1827, p. 263, pl. xxxvii, figs. 1, 2; de Blainville, 1828, p. 565; Gervais, 1836, p. 629.

Clepsina complanata, de Filippi, 1837, p. 27; Brightwell, 1842, p. 14, pl. i, fig. 20 (coloured, and named in error *C. hyalina*; figs. 18 and 19 represent *C. heteroclita* and not *C. complanata* as named).

Glossipora tuberculata, Thompson, 1841, p. 482, and 1856, p. 245 (occurrence in Ireland).

Glossiphonia sexoculata, Moquin-Tandon, 1846, p. 353, pl. xii, figs. 1—6 (coloured), 7—21 (anatomy); Thompson, 1846, p. 390 (recorded from Lough Neagh, Ireland); Johnston, 1865, p. 51; Dutilleul, 1887 a, p. 128 (dorsal organ, etc.); R. Blanchard, 1892 c, p. 178, figs. 1, 2; R. Blanchard, 1893 b, p. 3; R. Blanchard, 1893 d, p. 92 (in Norway); Bolsius, 1894 b, p. 292 *et seq.*; Bolsius, 1895, p. 159, pl. ix (Gregarines in intestine); Bayer, 1898, p. 648 *et seq.* (sense organs).
Glossiphonia cimiformis, Baird, 1869, p. 317.
Clepsine elegans, Verrill, 1872, p. 684.
Clepsine pallida, Verrill, 1872, p. 684.
Clepsine patelliformis, Nicholson, 1873, p. 494.
Clepsine sexoculata, Apáthy, 1888 a, p. 154, etc., pl. viii, fig. 2 (head); Apáthy, 1888 b, p. 791, etc.; Bürger, 1902, p. 525 *et seq.*, pls. xxx—xxxii (development).
Glossosiphonia complanata, Blanchard, 1894 b, p. 27, figs. 2, 3; Johansson, 1909, p. 74, figs. 125, 126.
Glossiphonia complanata, Scharff, 1898, p. 192.
Glossiphonia elegans, Castle, 1900 a, p. 46, pl. vii, pl. ii, fig. 5, pl. iii, fig. 11.

Diagnosis. Body ovate-elliptical, of a firm and cartilaginous consistency, with a somewhat rough surface, more or less transparent, greenish or brownish, very variable in colour and markings.

Dorsal surface with two longitudinal, dark brown, interrupted lines, arising in somite V, in an outer paramedian position and, typically, with six longitudinal rows of yellowish spots, which occur on the first ring of each somite and correspond to the inner paramedian, intermediate and outer paramarginal sense organs and papillae. These spots may be rounded and compact aggregations of pigment cells or more or less spread out into irregular confluent blotches; the intermediate and, less frequently, the paramarginal series may be absent. The series of spots corresponding to the inner paramedian (and largest) papillae are the most constant and occasion the interruptions in the dark brown lines.

Two interrupted brown lines occur on the ventral surface. These are wider apart and less conspicuous than the dorsal pair and rarely traverse the entire length of the body.

68 rings. Somites I—III and XXVI—XXVII uniannulate, IV, XXIV and XXV biannulate, the nineteen somites V—XXIII complete with three rings.

The six eyes are disposed in two close parallel rows (on the inner paramedian lines). The first and smallest pair, which occasionally is absent, occurs in somite II but may be shifted somewhat further back and appear between somites II and III or in the anterior part of somite III. The second and larger pair lies in the posterior part

of somite III and the third pair is situated in the first ring of somite IV.

The second ring of somite IV, often imperfectly divided from the first ring of somite V, forms the posterior boundary of the anterior sucker. The mouth opens in somite II.

Male genital orifice between rings 25 and 26, that is, between the second and third rings of somite XI; female orifice two rings behind the male, between the first and second rings of somite XII. Testes 10 pairs.

Eggs attached to some foreign body and brooded over by the parent.

Anus behind ring 67, separated from the posterior sucker by the sixty-eighth and last ring, which is incomplete.

Length, at rest, 15—20 mm.; width, at rest, 5—9 mm. Length, fully extended, up to 35 mm.

Distribution, Food, Varieties. A sluggish species very readily rolling itself up into a ball (like *Oniscus*) when disturbed; producing eggs during April and May, for a period of not much more than four weeks and rearing only one brood (Whitman, 1878, p. 10).

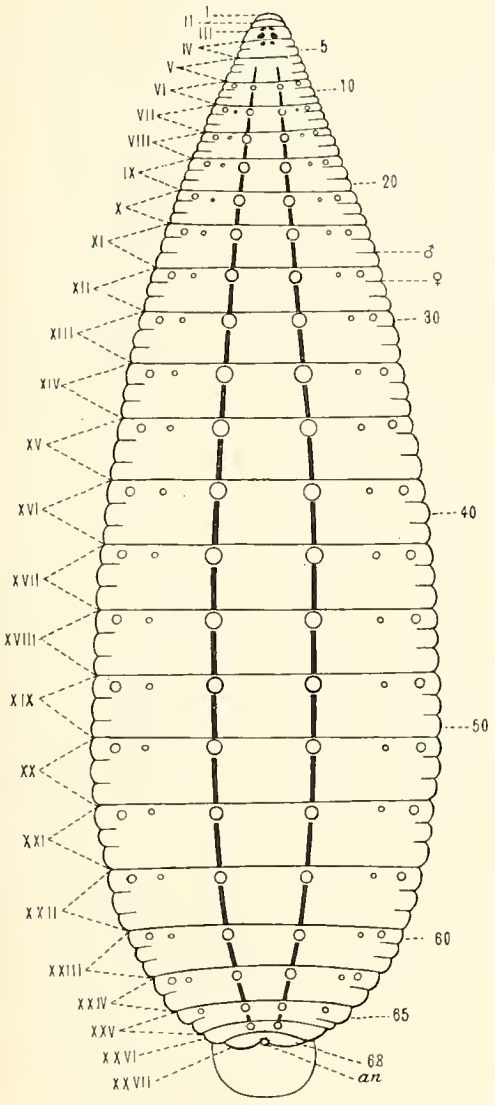
Very common in the British Islands, in running and stagnant water, among aquatic plants and upon or beneath stones. It is parasitic chiefly upon *Limnea* and *Planorbis* but also attacks other fresh-water molluscs, the larvae of *Chironomus* ("bloodworms") and probably aquatic annelids. Houghton (1865, p. 87) described three varieties of this leech and my own observations largely confirm his results. English examples resolve themselves more or less into the three following forms:

A. Body greenish, the six dorsal rows of yellow spots well marked (the typical form).

B. Body brown, the brown pigment being disposed in the form of minute longitudinal and transverse striae; the six dorsal rows of spots more or less dispersed into irregular blotches.

C. Body olive brown or brown, the pigment not striated, the longitudinal dark brown lines less distinct; without spots in regular rows; the anterior pair of eyes sometimes absent.

I have found A and B about equal in frequency; C is of rarer occurrence and approaches the variety *concolor* described by Apáthy from the Danube, and considered by him as a separate species. The matter requires further investigation.



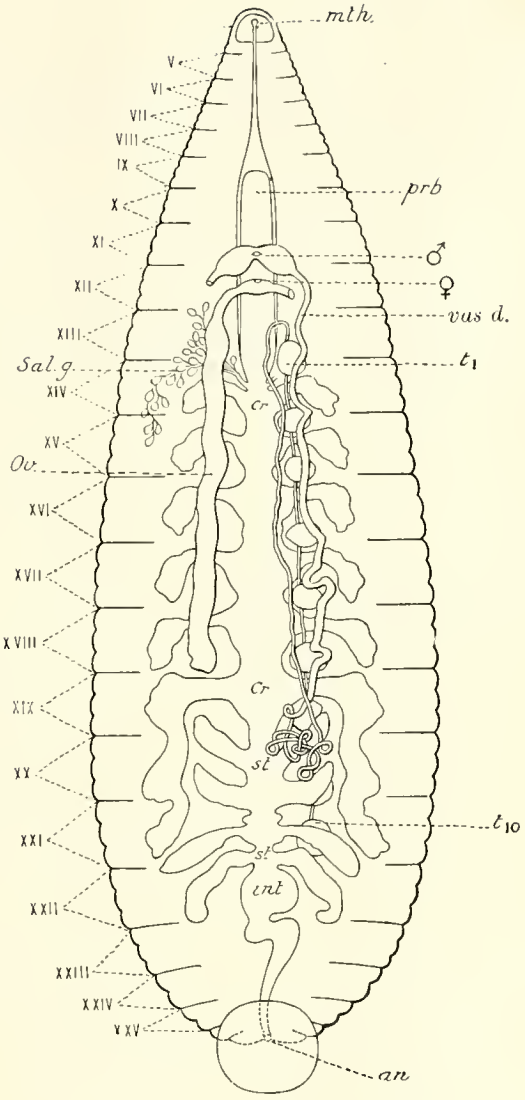


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Fig. 9. *Glossosiphonia complanata.*

Fig. 10. *Glossosiphonia complanata.*

Fig. 9. *Glossosiphonia complanata.* Diagram showing dorsal pattern and annulation. Somites numbered in Roman numerals and rings in figures.

Fig. 10. The same. Ventral view of alimentary and reproductive systems. *mth.* Mouth. *prb.* Proboscis. *Sal. g.* Salivary glands. *Cr.* Crop. *st.* Stomach. *int.* Intestine. *an.* Anus. *vas d.* Vas deferens. *t* 1, *t* 10, testes of the first and tenth pairs. *Ov.* Ovary. (Schematic.)

Genus: **Helobdella**, *R. Blanchard*, 1896.

Synonymy:

Glossiphonia, Johnson, 1816 (partim). *Clepsine*, Savigny, 1817 (partim).

Small Glossosiphonidae with one pair of eyes. Body generally without papillae. Complete somite composed of three rings. Crop with six pairs of simple lateral caeca, the last and longest pair reflected posteriorly.

This genus has been established by Professor R. Blanchard (1896 a, p. 4) in order to admit *Glossosiphonia stagnalis* (Linnaeus, 1758) and a number of closely allied species which are found for the most part in South America. Johnson's genus *Glossosiphonia* is thus reduced to a more homogenous group containing only six-eyed forms.

Helobdella stagnalis, *Linnaeus*, 1758.

Plate XIV, Figs. 13—17. Text Fig. 8 (p. 157).

Synonymy and Literature:

Hirudo bioculata, Bergman, 1757, pl. vi, figs. 9—11; O. F. Müller, 1774, p. 41; O. F. Müller, 1776, p. 220; Gmelin, 1788, p. 3096; Ure, 1793, p. 234; Schrank, 1804, p. 161; Braun, 1805, pp. 53—55, pl. vi, figs. 1—5 (coloured); Bosc, 1802, p. 256; Stewart, 1817, p. 357; Carena, 1820, p. 302, pl. xii, fig. 21 (coloured); Bruguière, 1824, p. 132, pl. li, figs. 9—11.

Hirudo stagnalis, Linnaeus, 1758, p. 649; Linnaeus, 1761, No. 2081; Weser, 1765, p. 44; Turton, 1806, p. 69; Turton, 1807, p. 129; Pennant, 1812, p. 71; Dalyell, 1853, p. 36, pl. iv, figs. 1—16 (coloured).

Hirudo pulligera, Daudin, 1800, p. 19, pl. i, figs. 1—3; de Blainville, 1827, p. 266.

Hirudo circulans, Sowerby, 1806, p. 31, pl. lxxvi; Turton, 1807, p. 129; Pennant, 1812, p. 72; Johnson, 1816, p. 27.

Helluo (*Hirudo*) *bioculata*, Oken, 1815, p. 368.

Glossiphonia perata, Johnson, 1816, p. 26.

Glossopora punctata, Johnson, 1825, p. 50, pl. xvii, figs. 11—13.

Erpobdella bioculata, de Blainville (in Lamarck), 1818, v, p. 296; de Blainville, 1827, p. 265.

Clepsine bioculata, Savigny, 1822, p. 119; Carena, 1820; Moquin-Tandon, 1826, p. 102, pl. iv, fig. 2; Leydig, 1849, pl. iii, figs. 9—11; Diesing, 1850, p. 448; Metschnikoff, 1871, p. 505 (development); Whitman, 1878, p. 2 *et seq.* (development); Apáthy, 1888 a, p. 154 *et seq.*, pl. viii, figs. 4 and 10 (head region); Apáthy, 1888 b, p. 790 *et seq.* (diagnosis); Leuckart, p. 610, fig. 260 B (proboscis); Oka, 1894, p. 81 *et seq.* (sinus system, nephridia).

Glossopora bioculata, Fleming, 1822, p. 604.

Hirudo stagnorum, Derheims, 1825, pp. 10 and 20.

Clepsine sowerbyi, Moquin-Tandon, 1826, p. 107; Diesing, 1850, p. 451.

Hirudo (*Glossobdella*) *pulligera*, de Blainville, 1827, p. 266, pl. xxxvii, fig. 6.

Glossobdella bioculata, de Blainville, 1828, p. 565, pl. xxxvii, figs. 3, 3 a and 3 b; Gervais, 1836, p. 629, pl. ccxi, fig. 9.
Erpobdella stagnalis, Templeton, 1836, p. 235.
Clepsina stagnalis, de Filippi, 1837, p. 27; de Filippi, 1839, p. 6; Brightwell, 1842, p. 14.
Glossiphonia bioculata, Moquin-Tandon, 1846, p. 366, pl. xiii, figs. 16—26; Thompson, 1846, p. 390, footnote (recorded from Lough Neagh, Ireland); Houghton, 1861, p. 33 *et seq.*, pl. iii, figs. 5 and 8 (cervical plate with parasitic *Epistylis*); Houghton, 1865, p. 82 *et seq.*, pl. i, figs. 7—9 (coloured); Ninni, 1889; R. Blanchard, 1893 d (in Norway); R. Blanchard, 1893 k, p. 43 (occurrence in Azores).
Glossiphonia circulans, Moquin-Tandon, 1846, p. 384.
Glossipora bioculata, Thompson, 1856, p. 425 (occurrence in N. Ireland).
Clepsine filippi, Polonio, 1863.
Clepsine modesta, Verrill, 1872, p. 679.
Clepsine submodesta, Nicholson, 1873.
Clepsine viridissima, Picaglia, 1877.
Glossosiphonia stagnalis, R. Blanchard, 1894 b, p. 25 (diagnosis).
Helobdella stagnalis, R. Blanchard, 1896 a, p. 4 (occurrence in S. America); Johansson, 1909, p. 76, figs. 131, 132.
Glossiphonia stagnalis, Scharff, 1898, p. 191; Castle, 1900 a, p. 21, pl. i, figs. 1—3 and pl. ii, fig. 4; Evans, 1905, p. 215 (occurrence in Scotland).
Helobdella bioculata, Beyer, 1898, p. 648 *et seq.* (sense organs).

Diagnosis. Body elliptic-lanceolate, much flattened, without papillae, more or less transparent, pale gray, often with a greenish, yellowish or brownish tinge, finely speckled with black. 68 rings.

The two eyes are closely approximated (in an inner paramedian position) and lie upon the third ring or between rings 2 and 3.

A rounded, brownish chitinous plate is situated in the dorsal median line, between rings 12 and 13.

The male genital orifice lies between rings 24 and 25; the female orifice is situated one ring behind the male, between rings 25 and 26.

Testes, 6 pairs.

Eggs attached to the ventral surface of the parent.

The anus lies behind ring 67 and is separated from the posterior sucker by the sixty-eighth and last ring, which is incomplete.

Length, at rest, 8—12 mm.; width, at rest, about 4 mm. Length, fully extended, 23—26 mm.

The length of the body in extreme extension may be as much as twelve times the width.

Distribution, Food, etc. A small and active species common, and in some places abundant, in the British Islands, in lakes, ponds, ditches and sluggish streams, chiefly among aquatic plants.

Parasitic largely upon gasteropods, but preys upon a variety of other hosts. The larvae ("bloodworms") of several species of *Chironomus*, which contain haemoglobin, are a favourite source of food and impart a scarlet colour to its crop. The whole contents of the body of the larva are extracted, leaving the transparent integument entire.

Moore (1901) states that it attacks small annelids, injured fish and frogs, and Blanchard (1894 b) records it from the bodies of newts.

Its range extends throughout the greater part of Europe into western Asia. It is found in Canada (Nicholson, 1873, = *Clepsine submodesta*) and in the United States from the Atlantic to the Pacific coast (Verrill, 1872; Blanchard, 1900; Moore, 1901). In South America it has been recorded by Blanchard (1896) from Paraguay and from the western slopes of the Andes.

Dorsal plate. The dorsal chitinous plate which forms a characteristic feature of this species, has been shown by Apáthy (1888 d, p. 202) to be the survival of an embryonic attachment gland, giving off a tuft of tenacious chitinous threads, like a byssus, which hardens in the water and serves, before its suckers are developed, to fix the embryo to the venter of the parent. The somewhat rough and hollow surface of this structure is a favourite place of attachment for colonies of *Epistylis*.

A second example of a dorsal plate has been described by Blanchard (1900, p. 9) in the South American species, *Helobdella scutifera*.

A provisional attachment gland, which leaves scarcely perceptible traces in the adult, occurs, according to Apáthy (*loc. cit.*) in the embryo of *Glossosiphonia heteroclita* and inconspicuous rudiments of similar organs have been described in several other species (Apáthy, *loc. cit.*; Nusbaum, 1885, p. 181; Dutilleul, 1887 a, p. 128).

For the general anatomy of *H. stagnalis* see Castle (1900), p. 21 *et seq.*

Sub-order II. ARHYNCHOBDELLAE.

Fresh-water and terrestrial Hirudinea with red blood, without an exsertile proboscis, generally with jaws. Anterior sucker with a ventral aspect, not distinct from the body. Seventeen pairs of nephridia.

Family I. GNATHOBDELLIDAE.

With five, or rarely with four, pairs of eyes and, except in the Semiscolecinae, with three denticulate jaws. Eggs enclosed in a free spongy cocoon which is deposited above the water line.

This family, which includes the typical ten-eyed blood-sucking leeches, has been divided by Blanchard (1896 a, p. 9) into the following sub-families:—(1) The *Haemadipsinae*, comprising the blood-sucking land-leeches; (2) the *Hirudininae*, discussed below and (3) the *Semiscolecinae*, a small group of amphibious forms without jaws, possessing distinct affinities with the *Herpobdellidae*. With the exception of one species of *Haemadipsinae*, the curious *Xerobdella lecomtei* (von Frauenfeld, 1868), found in the Austrian Alps, the first and third of these sub-families are not represented in Europe.

Sub-family. Hirudininae.

With five pairs of eyes and with denticulate jaws. Complete somite formed of five rings. The nephridial pores open near the margins of the body upon the ventral surface.

Blanchard (1896 b) divides this group into two series based upon characters exhibited by the teeth and jaws.

Series 1. **Distichodonta.**

Jaws without papillae and armed with two rows of infrequent, blunt, irregular teeth.

Genus: **Haemopis**, *Savigny*, 1822.

Synonymy:

Aulastoma, Moquin-Tandon, 1826. *Hirudo* (*Pseudobdella*), de Blainville, 1827. *Hirudo* (*Hippobdella*), de Blainville, 1827. *Pseudobdella*, de Blainville, 1828. *Aulacostomum*, Grube, 1850. *Aulostomum*, Polonio, 1860.

Crop with one pair of elongate, lateral caeca reflected posteriorly. Genital openings usually separated by five rings. Upper lip of anterior sucker not divided inferiorly by a longitudinal groove.

Haemopis sanguisuga, *Linnaeus*, 1758.

Plate XV, Figs. 39—41. Text Figs. 11 and 12 (pp. 167 and 170).

Synonymy and Literature:

Rossaglen, Aldrovandus, 1602, p. 722.

Horseleech, Mouffet, 1634, p. 323, woodcut.

Hirudines venenatae, Horse-Leeches, Sibbald, 1683, p. 34.

Hirudo maxime apud nos vulgaris—the Horse-Leech or Bloodsucker, Ray, 1710, p. 3.

Hirudo nigra, abdomine plumbeo—the Horse-Leech, Hill, 1752, p. 16.

Hirudo sanguisuga, Merret, 1667, p. 207 ; Bergman, 1757, pl. vi, figs. 3, 4 ; Linnaeus, 1758, p. 649 ; Gisler, 1758, p. 95 ; Linnaeus, 1761, No. 2078 ; Müller, 1774, p. 38 ; Müller, 1776, p. 220 ; Barbut, 1783, p. 20, pl. ii, fig. 6 ; Gmelin, 1788, p. 3095 ; Bosc, 1802, p. 246 ; Turton, 1806, p. 68 ; Turton, 1807, p. 129 ; Watson, 1812, p. 13 ; Pennant, 1812, p. 70 ; Johnson, 1816, p. 30 ; Stewart, 1817, p. 356 ; Cuvier, 1817, p. 532 ; Carena, 1820, p. 286, pl. xi, figs. 7, 8, 12, 23, 25 and 26 ; Bruguière, 1824, p. 132, pl. li, figs. 3, 4 ; Audouin, 1825, p. 8 ; Derheims, 1825, pp. 9 and 19 ; Stark, 1828, p. 356 ; Templeton, 1836, p. 235 ; Bowerbank, 1845, p. 301, pl. 18 (cocoon) ; Dalyell, 1853, p. 2, pl. iii, figs. 1—10 (coloured).

Hirudo gulo, Braun, 1805, p. 12, pl. i, figs. 1—7 (coloured).

Hirudo vorax, Johnson, 1816, p. 62 ; Pelletier et Huzard, 1825, p. 121.

Hirudo sanguisorba, de Blainville (in Lamarck), 1818, p. 291 ; Fleming, 1822, p. 604 ; Milne-Edwards (in Lamarck), 1835, p. 521.

Haemopis nigra, Savigny, 1820, p. 116 ; Brandt et Ratzeburg, 1829, pl. xxix, B. figs. 12—17 (anatomy).

Haemopis sanguisorba, Savigny, 1820, p. 115 ; Brightwell, 1842, p. 12 ; Quekett, 1843 (not Diesing, 1850).

Haemopis luctuosa, Savigny, 1822, p. 116.

Haemopis lacertina, Savigny, 1822, p. 117.

Hirudo carnivora, Brossat, 1822, p. 34 (?).

Aulastoma nigrescens, Moquin-Tandon, 1826, p. 124, pl. vi, fig. 3 (bad) ; Cuvier, 1836, p. 215 ; Williams, 1851, p. 238.

Hirudo (*Pseudobdella*) *nigra*, de Blainville, 1827, p. 249.

Hirudo (*Hippobdella*) *sanguisuga*, de Blainville, 1827, p. 254 ; Gervais, 1836, p. 638, pl. ccxi, fig. 4, a and d.

Pseudobdella nigra, de Blainville, 1828, p. 560, pl. xxxv, fig. 1.

Hippobdella sanguisorba, de Blainville, 1828, p. 561, pl. xxxv, fig. 2.

Hirudo (*Pseudobdella*) *vorax*, Gervais, 1836, p. 628, pl. ccxi, figs. 6 and 6 a and b.

Haemopis ornata, de Filippi, 1837, p. 25, fig. 14.

Aulastoma gulo, Moquin-Tandon, 1846, p. 313, pl. v, figs. 1—6 (coloured) ; Chworostansky, 1886, p. 446 (genital organs) ; Apáthy, 1888 a ; Apáthy, 1888 b ; Apáthy, 1889 a, p. 267 ; Graf, 1894 b.

Haemopis vorax, de Filippi, 1837, p. 25 ; Leydig, 1849 b, p. 16, pl. iii, fig. 4 (anatomy) ; (not Moquin-Tandon, 1826 b, p. 108).

Haemopsis vorax, Johnston, 1846, p. 442 ; Thompson, 1856, p. 427 (in Ireland).

Haemopsis nigra, Johnston, 1846, p. 442.

Haemopis sanguisuga, Hardy, 1850, p. 96 ; Hertwig, 1877, p. 2 *et seq.* (development) ; R. Blanchard, 1892 e, p. 3, figs. 1, 2 ; *ibid.*, 1893 a, p. 25 ; *ibid.*, 1893 b, p. 4 ; *ibid.*, 1893 d, p. 92 (in Sweden) ; *ibid.*, 1893 i, p. 3 (in Palestine) ; *ibid.*, 1894 b, p. 48 ; Scharff, 1898, p. 193 (in Ireland) (not Moquin-Tandon, 1846, p. 318).

Aulostomum gulo, Diesing, 1850, p. 461 ; Polonio, 1860 ; Schneider, 1880, pp. 19 and 256.

Aulacostomum gulo, Grube, 1851, p. 110 ; Grube, 1871, p. 97, pl. iii, fig. 7 (variety).

Aulostomum italicum, Polonio, 1860.

Aulostoma gulo, Johnston, 1865, p. 46.

Aulostoma nigra, Johnston, 1865, p. 46.

Aulastomum gulo, Rhode, 1891, p. 1 *et seq.* (nervous system); Retzius, 1891, p. 1 *et seq.* (nervous system); Leuckart, 1894, p. 617, fig. 264 (pharynx).

Diagnosis. Body soft and flaccid, flattened, attenuated anteriorly, bluntly rounded posteriorly, the sides more or less parallel from the clitellum to somite XXIII. It is less contractile than *Hirudo medicinalis* and incapable of assuming the form of an olive. *Very young individuals* are grayish, finely speckled above and below with black and bear upon the dorsal surface a geometrical pattern of which the most

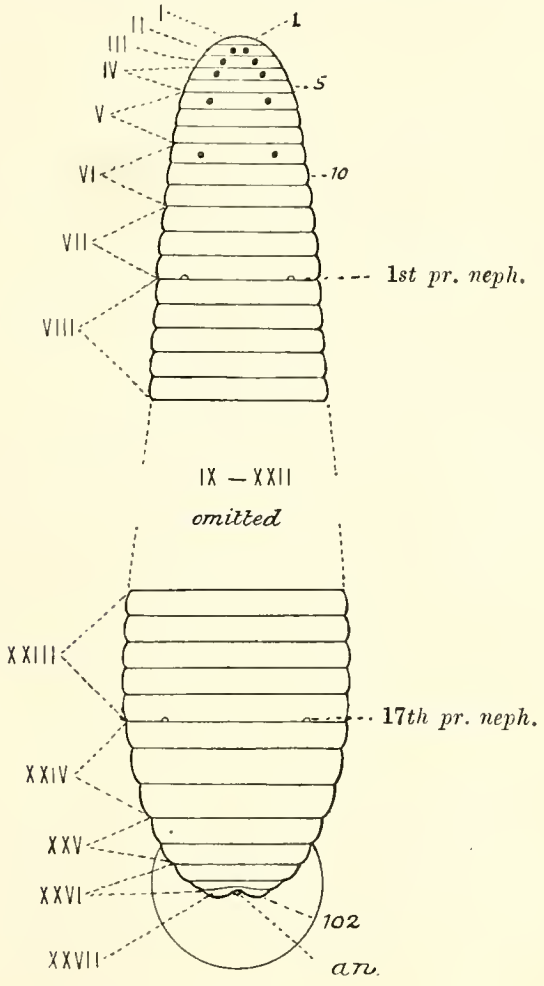

Fig. 11. *Haemopis sanguisuga*. Diagram of anterior and posterior extremities. Somites numbered in Roman numerals, rings in figures. The 5 pairs of eyes are indicated by black dots. 1*st pr. neph.*, 17*th pr. neph.*, position (on ventral surface) of first and seventeenth pairs of nephridiopores. *an.* Anus. (Adapted from Whitman.)

conspicuous feature consists of a pale median, longitudinal band, margined with black, alternately wide for the length of three rings and narrow for the length of two rings. The constricted portions of this band correspond to rings 3 and 4 of each somite and the dilated portions traverse rings 5, 1 and 2 of contiguous somites. *Adults* are blackish green, olive green, yellowish green or brownish, paler and often bright green ventrally, and more or less densely flecked with black above and below. The geometrical pattern seen in young individuals is almost or entirely obliterated. The pale, median dorsal band sometimes may be traced in favourable examples of light colouration; its black margins frequently persist in the form of two somewhat broken and irregular black stripes.

102 rings. Rings 6 and 7 are united ventrally to form the posterior margin of the anterior sucker. Rings 8 and 9 are also fused on the ventral side. The last ring (102), which is pierced by the large anus, may show signs of sub-division: Whitman (1886, pp. 371—372) assigns to it the value of two rings.

Somites I, II, III and XXVII, uniannulate; IV, XXV and XXVI biannulate; V, VI, VII and XXIV triannulate; the sixteen somites VIII—XXIII are complete with five rings.

The five pairs of eyes lie respectively in rings 2, 3, 4, 6 and 9. [*Note.* The disposition of the eyes and the external metamerism, except in the case of somite XXVII, are the same as in *Hirudo medicinalis.* In the latter leech somite XXVII is biannulate and consequently its body possesses one ring more than *Haemopis sanguisuga.*]

Oesophagus with twelve longitudinal plications, the three largest ending anteriorly in jaws.

Each jaw is armed with 11—18 pairs of irregular, blunt teeth (R. Blanchard).

The male genital orifice is situated between rings 31 and 32, that is between the second and third ring of somite XI, or upon ring 32; the female orifice lies between rings 36 and 37, that is between the second and third rings of somite XII, or upon ring 37. One or both of these orifices may thus be shifted forwards by the space of half a ring; normally they are separated by the space of five rings.

Size. The following dimensions apply to average, medium-sized individuals:

In contraction, 25—35 mm. long and 10—12 mm. wide. Fully extended 90—100 mm. long and 5—6 mm. wide. Individuals occur,

however, which can extend to a length of nearly six inches. The largest example I have met with measured:—contracted, at rest, 40 mm. long and 15 mm. wide. Fully extended, 147 mm. long and 7 mm. wide.

Distribution, Food, etc. This well-known leech occurs throughout the greater part of Europe and its range extends, according to Blanchard (1893 a, p. 25; 1893 i, p. 3) into Transcaucasia and Syria.

In England, Scotland and Ireland it is common, chiefly in the mud at the bottom of sluggish streams and ponds. It leaves the water voluntarily in order to deposit its cocoons and probably also in pursuit of its prey.

Haemopis sanguisuga is carnivorous, devouring piecemeal earthworms and, according to several authorities, molluscs, aquatic larvae, tadpoles, and small or wounded fish and frogs. It attacks *Herpobdella*, *Trocheta* and, under the influence of hunger, even members of its own species; Ébrard counted it among the enemies of *Hirudo medicinalis*. Probably as Dalyell states, "few animal substances are rejected" by this voracious species.

The blunt teeth of this leech cannot pierce the human skin. Its character as a blood-sucker appears to be due to confusion with *Hirudo medicinalis* and *Limnatis nilotica*, and to the same cause must be attributed its alleged habit of lurking in drinking places and crawling into the mouths and nasal apertures of horses and cattle. Early writers frequently refer to the "horse-leech or bloodsucker" when the medicinal leech is intended (e.g. Burton: *Anatomy of Melancholy*, Pt. 2, S. 5, Mem. 3, s. 1).

The term "horse-leech" or "cattle-leech" has been applied to more than one species of *Limnatis*, in which the above habit is strongly developed. *Limnatis nilotica*, the only other "horse-leech" found in Europe, occurs in Italy and Spain and is frequent in North Africa and parts of Western Asia. This dangerous parasite is sometimes inadvertently swallowed by human beings and by cattle[1].

Varieties. A number of colour-varieties of this species have been described, the most important of which, such as *H. ornata*, de Filippi (1837), are dependent on the amount of persistence shown by the primitive dorsal geometrical pattern seen in young individuals.

[1] A description of accidents of this nature, together with a note on this leech, have already appeared in the pages of this Journal. [Masterman, *Parasitology*, I. p. 186; Harding, *ibid.* p. 282.]

The blackish green pigment generally prevalent on the dorsal surface of adults is more soluble in alcohol than the black markings which it frequently obscures. In specimens which have been submitted to prolonged immersion in alcohol it becomes entirely dissolved and the primitive pattern again is rendered more or less evident. Blanchard (1892 e, p. 3, fig. 1) gives a figure of an adult specimen of this kind in the Dresden Museum, in which the pattern is almost perfectly preserved.

Confusion of species. Moquin-Tandon confused the species now under consideration with *Limnatis nilotica,* Savigny, 1822. In the two editions (1826 and 1846) of his classical monograph we have a far from adequate description of *Limnatis nilotica* and also what is, in its essential characters, nothing more than a second description of the same species,

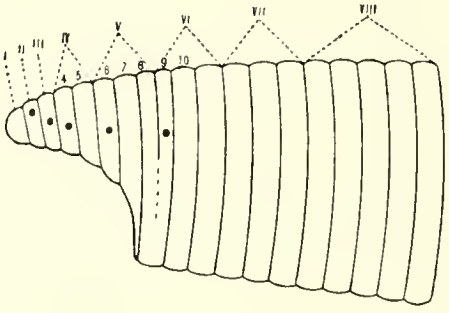

Fig. 12. Side view of head region in *Hirudo medicinalis* and *Haemopis sanguisuga*. Somites numbered in Roman figures, Rings in italics. The eyes are indicated by black dots.

under the names *Haemopis vorax*, 1826, and *Haemopis sanguisuga*, 1846. When therefore this writer turns to the true *Haemopis sanguisuga* it becomes necessary to provide for it a new name, and accordingly he establishes for its reception a new genus *Aulastoma* (1826) and, in the second and best known edition of his work (1846), we find it described as *Aulastoma gulo.*

Thus arose the erroneous idea that *Aulastoma gulo* and *Haemopis sanguisuga* were two distinct species.

Whitman (1886) and Apáthy (1888 a) rejected *Haemopis sanguisuga* as described by Moquin-Tandon, but it remained for Blanchard (1894 b, p. 43) to give a complete solution of the difficulty.

Series 2. **Monostichodonta.**

Jaws with or without papillae and armed with a single row of numerous sharp teeth.

Genus: **Hirudo**, *Linnaeus*, 1758.

Synonymy:

Sanguisuga, Savigny, 1822. *Iatrobdella*, de Blainville, 1827.

Jaws devoid of papillae and armed with less than 100 *sharp teeth. Crop with ten pairs of lateral caeca of which the last and longest pair is reflected posteriorly. Genital openings separated by five rings. Upper lip of anterior sucker not divided inferiorly by a longitudinal groove.*

Hirudo medicinalis, *Linnaeus*, 1758.

Plate XV, Figs. 36—38. Text Figs. 12 and 13 (pp. 170 and 174).

Synonymy and Literature:

Hirudo major et varia, Gesner, 1558, p. 503.
La Sangsue, Rondelet, 1558, p. 169.
Hirudo varia, Aldrovandus, 1602, p. 763.
Hirudo minor variegata, Muralto, 1685, p. 579.
Bloetsuyger, Swammerdam, 1737, p. 62.
Hirudo depressa, nigra ; abdomine subcinereo, Linnaeus, 1746, p. 365.
Hirudo nigreseens, flavo-variegata—the Common Leech, Hill, 1752, p. 16, pl. ii.
Hirudo medicinalis, Ray, 1710, p. 3 ; Dillenius, 1719, Cent. 7, pl. v, figs. 1—4 ; Bergman, 1757, p. 308, pl. vi, figs. 1, 2 ; Linnaeus, 1758, p. 649 ; Gisler, 1758, p. 95 ; Salomon, 1760, p. 35 ; Linnaeus, 1761, No. 2079 ; Weser, 1765, p. 41 ; Pennant, 1766, p. 36 ; Linnaeus, 1767, p. 1079 ; Shaw, 1769 ; O. F. Müller, 1774, p. 37, No. 167 ; O. F. Müller, 1776, p. 219, No. 2658 ; Barbut, 1783, p. 19, pl. ii, fig. 5 ; Gmelin, 1788, p. 3095 ; Cuvier, 1798, p. 632 ; Bosc, 1802, p. 245, pl. viii, fig. 6 ; Draparnaud, 1803, p. 31 ; Turton, 1806, p. 68 ; Turton, 1807, p. 129 ; Pennant, 1812, p. 69 ; Oken, 1815, p. 368 ; Cuvier, 1817, p. 532 ; Stewart, 1817, p. 356 ; de Blainville, in Lamarck, 1818, p. 291 ; Bojanus, 1819, p. 468, pl. f, figs. 1—5 ; Carena, 1820, p. 279, pl. xi, figs. 1, 2 ; Delle Chiaje, 1823, p. 47 ; Bruguière, 1824, p. 131, pl. li, figs. 1, 2 ; Fleming, 1822, p. 604 ; Leach, 1824, p. 451, pl. xxvi ; Payraudeau, 1826, p. 17 ; Fischer, 1827 ; L. G. Müller, 1830 ; Milne-Edwards, in Lamarck, 1835, p. 520 ; Moquin-Tandon, 1846, p. 327, pls. vii—xi ; Johnston, 1846, p. 442 ; Diesing, 1850, p. 465 ; Grube, 1851, p. 109 ; Dalyell, 1853, p. 26, pl. iii, fig. 11 (coloured) ; Ranke, 1875, p. 152, figs. 1—12 (eyes) ; Hermann, 1875 (nervous system) ; Ollson, 1875, p. 3 ; Bourne, 1880, p. 283, pls. xxiv and xxv (nephridia) ; Lankester, 1880 a, p. 85 ; Lankester, 1880 b, p. 305 (intra-epithelial capillaries) ; Lankester, 1880 c, p. 307 (connective tissue) ; Schneider, 1880, pp. 19

and 256 (embryology); Carlet, 1883 a, p. 448 and 1883 b (fixation of suckers); Schultze, 1883, p. 87, figs. 15, 16 (nephridia); Bourne, 1884 (nephridia, etc.); Whitman, 1884, p. 76 (external morphology); Haycraft, 1884, p. 478 (secretion of an anticoagulin); Bertelli, 1887, p. 284 (salivary glands); Apáthy, 1888 a; Apáthy, 1888 b; Apáthy, 1889 a, p. 267; Griffiths, 1889, p. 346 (nephridia); Biedermann, 1891, p. 434 (nerve fibres); Retzius, 1891 (nervous system); Henking, 1892, p. 319, pl. xxxiii (digestive tract); R. Blanchard, 1893 k, p. 44 (in Syria); R. Blanchard, 1894 b (in Italy, descr.); Croockewit, 1894, p. 427 (jaws); Bürger, 1894, p. 440 (embryology); Graf, 1894 a, p. 485 (nephridia); Bertelli, 1896, p. 147, pl. ii (pharyngeal glands); Apáthy, 1897, p. 37, pls. iv—vi (glands): Scharff, 1898, p. 193 (in Ireland); Goodrich, 1899, p. 477, pls. xlii—xliv (communication between vascular system and coelom); Schuberg, 1899 (reproductive organs); Havet, 1899, p. 73 *et seq.* (nervous system); Allen, 1902, p. 161 (topography of internal organs); Spiess, 1902, p. 548 (digestive tract); Livanow, 1903 (neuro- and myosomite); Spiess, 1903, p. 151 (digestive tract); Fage, 1904, p. 1450 (nephridia); Spiess, 1905 a, pp. 415 and 577, and 1905 b, pp. 333 and 506 (biliary pigments).

Hirudo vencsector, Braun, 1805, p. 24, pl. xi, figs. 1—9 (coloured).

Medicinal Leech, Kurzmann, 1819, p. 312.

Sanguisuga medicinalis, Savigny, 1822, p. 114; Savigny, 1826, p. 456; Risso, 1826, p. 428; Moquin-Tandon, 1826, p. 114, pl. v, fig. 2; Fischer, 1827, p. 440, figs. 1—4; Brandt et Ratzeburg, 1829, p. 238, pl. xxviii, figs. 3—17 and A—M (coloured, except figs. 10—17), pl. xxix A, figs. 1—58 (anatomy), pl. xxix B, figs. 1—11 (anatomy), and pl. xxx, figs. 5—25 (embryology, etc.); de Filippi, 1837, p. 26; Wedeke, 1842, p. 183; Wedeke, 1843, p. 296; Brightwell, 1842, p. 13.

Sanguisuga officinalis, Savigny, 1822, p. 330; Savigny, 1826, p. 457; Moquin-Tandon. 1826, p. 112, pl. v, fig. 1; Fischer, 1827, p. 441, figs. 5—10; Audouin, 1829, p. 109; Brandt et Ratzeburg, 1829, p. 237, pl. xxx, figs. 1 and A, B, C (coloured).

Hirudo provincialis, Carena, 1820, p. 282, pl. xi, figs. 4, 5; Brandt et Ratzeburg, 1829, p. 237 (syn. *S. officinalis*), pl. xxx, fig. 1* and M (coloured).

Hirudo verbana, Carena, 1820, p. 285, pl. ix, fig. 6.

Hirudo officinalis, Derheims, 1825, pp. 9 and 11.

Sanguisuga verbana, Moquin-Tandon, 1826, p. 117, pl. vi, fig. 1; Audouin, 1829, p. 109; Brandt et Ratzeburg, 1829, p. 235, pl. xxx, fig. 2 (coloured); de Filippi, 1837.

Sanguisuga carena, Risso, 1826, p. 429.

Sanguisuga obscura, Moquin-Tandon, 1826.

Iatrobdella (*Hirudo*) *medicinalis*, de Blainville, 1827, p. 254.

Iatrobdella medicinalis, de Blainville, 1828, p. 561, pl. xxxv, figs. 4 and 4 a—4 d, also pl. xxxvi, figs. 1—3 (varieties); Egidy, 1844, p. 113, figs. 62, 63.

Sanguisuga chlorogaster, Brandt et Ratzeburg, 1829, p. 238, pl. xxviii, figs. 1, 2.

Diagnosis. Body elongate, flattened, widest at about the sixteenth somite, tapering anteriorly and posteriorly, capable of contracting into the form of an olive (Moquin-Tandon).

Dorsal surface usually olive green, richly variegated with reddish brown, yellowish green, orange and black, and exhibiting an extremely

variable pattern based generally upon three pairs of reddish brown or yellowish, more or less distinct, longitudinal stripes often interrupted by black ocelli or spots occurring on the last ring of each somite.

Ventral surface usually yellowish green, more or less spotted with black, with a pair of black marginal stripes.

103 rings. Rings 6 and 7 are fused ventrally to form the posterior margin of the anterior sucker. Rings 8 and 9 are also united on the ventral side. Somites I, II, III and XXVII uniannulate; IV, XXV and XXVI biannulate, V, VI, VII and XXIV triannulate; the 16 somites VIII—XXIII are complete with five rings.

The male genital orifice lies between rings 31 and 32, that is, between the second and third rings of somite XI; the female orifice is five rings behind the male, between the second and third rings of somite XII.

The anus opens in ring 102 or between this and the preceding ring.

Length, in extreme extension, 100—125 mm.; in extreme contraction, 30—35 mm. Width, in extreme extension, 8—10 mm.; in extreme contraction, 15—18 mm.

Distribution. *Hirudo medicinalis* occurs in sluggish and stagnant waters in Europe and the adjacent parts of Asia. In Europe, where it was formerly abundant, it is now chiefly confined to the South and East. In Germany it is still found in the Island of Borkum, near Marksuhl, in Thuringia and perhaps also near Mieselstein, in Allgau (Johansson, 1909, p. 78).

It was at one time a common British species but was becoming less frequent as early as 1816. "Formerly," says Johnson (1816, p. 41), "this species was very abundant in our island; but from their present scarcity, owing to their being more in request among medical men, and to the rapid improvements which have of late years taken place in agriculture, particularly in the draining and cultivation of waste-lands, we are obliged to receive a supply from the Continent." In 1842, according to Brightwell (p. 13), it was found occasionally in the neighbourhood of Norwich, but was by no means common, and Johnston (1865, p. 49) states that the only British examples he had seen were the two in the British Museum Collection which he refers in his *Catalogue* to the variety *chlorogastra*.

In Scotland Dalyell (1853, p. 29) notes that medicinal leeches "of late years...had become scarce at the places previously affording them," and Thompson (1856, p. 427) states that, although becoming scarce, they

were still found in Ireland in 1849. There seems to be no doubt that this species is now extinct in the British Islands.

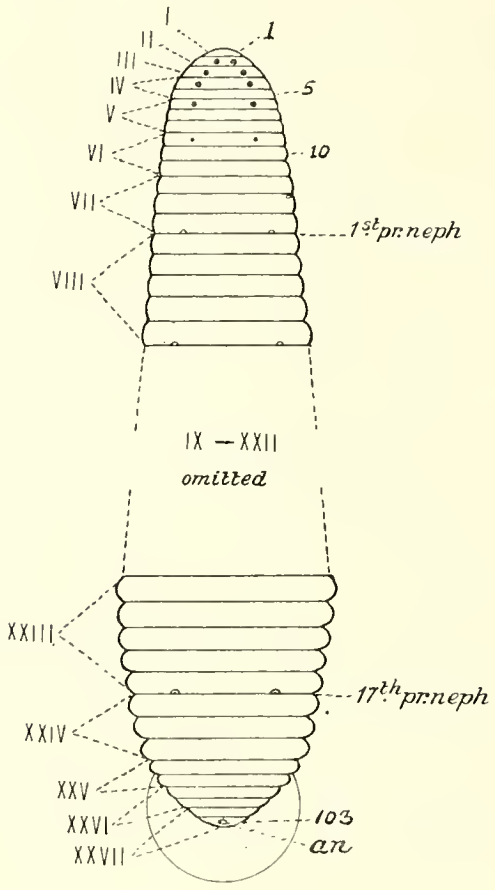

Fig. 13. *Hirudo medicinalis.* Diagram of anterior and posterior extremities. Somites are numbered in Roman numerals and rings in figures. The 5 pairs of eyes are indicated by black dots. *1st pr. neph.*, *17th pr. neph.*, position (on ventral surface) of first and seventeenth pairs of nephridiopores. *an.* Anus. (Adapted from Whitman.)

Leeches in Commerce. Although *Hirudo medicinalis* was known to the ancients it was not in great demand for use in phlebotomy until the beginning of the nineteenth century. The traffic in medicinal leeches soon assumed enormous proportions and reached its zenith in France in 1832, in which year, Ébrard tells us, nearly 57½ millions of these annelids were imported into that country. As the natural sources of supply failed, the artificial cultivation of leeches in special ponds became a profitable industry. For further information on the subject

of Hirudiniculture the curious reader is referred to the comprehensive monograph of Ébrard (1857).

Leech farming was never practised in this country, although according to Johnson (1816) and Dalyell (1853) "leech-gathering" appears to have been a not uncommon and somewhat remunerative calling in the earlier part of the last century. In Ireland Thompson (1856, p. 427) states that, in 1849, the medicinal leech (for which the Irish name was *dallog*) was still found in pools in the neighbourhood of Lough Mask and that "in summer the leech-gatherers sat with their legs in the water on which the creatures fasten and are thus obtained."

The indigenous supply was supplemented by large importations of leeches from abroad. In 1816 we employed "at least one hundred foreign leeches for every British leech" (Johnson). Brightwell, in 1842, refers to a dealer in Norwich who kept a stock of about 50,000 leeches in two large tanks. At this time leeches were imported in large sacks, sometimes but not always packed in damp grass, and the resulting mortality among them was very great.

Not infrequently consignments of the medicinal leech were adulterated with quantities of the innocuous horse-leech.

Hirudo medicinalis is not the only leech which has been used in phlebotomy. *Hirudo troctina* (Johnson, 1816), occurring in North Africa and in Southern Europe, where it is perhaps an introduced species, was largely imported at one time for medical uses.

The characteristic ocelli on the dorsal surface of the latter species earned for it in England the name of "trout leech" whilst the fact that large numbers were imported from Algiers, together with the supposed resemblance of its dorsal pattern to the uniform of a French dragoon, led to the name of "*le dragon d'Alger*" by which it was known to the foreign trade.

Several other species have been used for blood-letting in different countries. *Limnatis* (*Poecilobdella*) *granulosa* in India, *Haementaria officinalis* in Mexico, *Hirudo nipponia* in Japan (Whitman) and *Macrobdella decora* in the United States (Verrill) are or have been used in phlebotomy.

Varieties. A large number of colour-varieties of *Hirudo medicinalis* have been described. For descriptions and coloured illustrations of these the reader is referred to the works of Brandt and Ratzeburg (1829), Moquin-Tandon (1846) and particularly to the monograph of Ébrard (1857).

Family II. **HERPOBDELLIDAE.**

Synonym:

Nephelidae.

Non-parasitic, carnivorous Arhynchobdellae without denticulate jaws. Eyes, when present, generally eight in number, disposed transversely in two groups which are separated by one or more rings. Complete somite composed of 5—11 *often unequal rings. The nephridial pores open near the margins of the body on the ventral surface. Eggs deposited in flattened elliptical capsules which are attached to some foreign body.*

This family is represented in the British Islands by two genera, *Herpobdella* and *Trocheta.*

In the first genus the rings composing a complete somite are all of equal size. This is not the case however in the second of these genera where, as we shall see, the complete somite, in its primitive form, consists of a number of equal rings together with one ring, the fourth, which is only half the size of the others. This odd ring or *intercalated ring* is found in the complete somite of several genera of *Herpobdellidae* and according to its absence or presence Blanchard (1897, pp. 101—103) has divided this family into two series:—(1) The *Haplodesminae,* in which the complete somite is composed of a simple (ἁπλοῦς) chain (δεσμός) of equal rings, and (2) the *Epactodesminae,* in which the somite is complicated by the presence of an intercalated (ἔπακτος) ring. The intercalated ring is not always the smallest in the series; in *Dina* (R. Blanchard, 1892), a genus closely allied to *Herpobdella,* and represented in Europe, it is the largest ring in the somite and divided transversely.

Series 1. **Haplodesminae.**

Complete somite without an intercalated ring.

Genus: **Herpobdella,** *de Blainville,* 1818.

Synonymy:

Helluo, Oken, 1815 (not *Helluo,* Bonelli, 1813). *Erpobdella,* de Blainville, 1818. *Nephelis,* Savigny, 1822. *Hirudo* (*Erpobdella*), de Blainville, 1827. *Herpobdella,* R. Blanchard, 1894.

With four or three pairs of eyes, the first pair of which always occurs in somite II. Complete somite formed of five equal, undivided rings. The

clitellum, well marked during the breeding season, extends from the second ring of somite X to the second ring of somite XIII, inclusive.

The two species, *H. octoculata* and *H. atomaria*, which represent this genus in the British Islands have constantly been confused. The latter species generally has been considered to be merely a variety of *H. octoculata* and has shared in its synonymy. The synonymy of *H. atomaria* has been here given as far as it could be ascertained, but a number of references which may apply indiscriminately to either species will be found under *H. octoculata*.

Herpobdella octoculata, *Linnaeus*, 1758.

Plate XV, Figs. 42—44. Text Fig. 14 (p. 179).

Synonymy and Literature:

Hirudo tenuior et a cauda muris non multum diversa, Aldrovandus, 1602, p. 722.

Hirudo octoculata, Bergman (partim), 1756, p. 199; Bergman (partim), 1757, pl. vi, figs. 5—8; Linnaeus (partim), 1758, p. 649; Linnaeus (partim), 1761, No. 2080; Weser, 1765, p. 44; Linnaeus (partim), 1767, p. 1079; Turton, 1806, p. 69; Turton, 1807, p. 129; Blumenbach, 1807, p. 432; Pennant, 1812, p. 71; Blumenbach, 1825, p. 244; Derheims, 1825, p. 10.

Hirudo vulgaris, O. F. Müller (partim), 1774, p. 40; O. F. Müller (partim), 1776, p. 220; Gmelin, 1788, p. 3096; Bruguière, 1791, pl. li, figs. 5—8; Bosc, 1802, p. 256; Schrank, 1803, p. 161; Braun, 1805, p. 39, pl. iii, figs. 4—11 (coloured); Johnson, 1816, p. 33; Johnson, 1817, p. 21, pl. ix; Stewart, 1817, p. 356; Carena, 1820, p. 290; Johnson, 1825, p. 29, pl. ix.

Nondescript Leech, Ure, 1793, p. 236.

Helluo (Hirudo) octoculata, Oken, 1815, p. 367.

Erpobdella vulgaris, de Blainville (partim, in Lamarck), 1818, p. 296; Fleming, 1822, p. 604; (not Delle Chiaje, 1823, p. 49); de Blainville (partim), 1828, p. 564, pl. xxxvi, figs. 4 and 4 a—4 i; Milne-Edwards (in Lamarck), 1835, p. 528; Egidy, 1844, p. 134, fig. 64; Johnston, 1846, p. 439; Thompson, 1856, p. 425 (in Ireland); (not Verany, 1846).

Nephelis tessellata, Savigny (partim), 1822, p. 117.

Nephelis testacea, Savigny, 1822, p. 117; de Filippi, 1837.

Nephelis tessulata, Risso (partim), 1826, p. 431.

Nephelis vulgaris, Moquin-Tandon (partim), 1826, p. 125, pl. vi, figs. 4, 5; de Filippi, (partim), 1837, p. 28; Brightwell, 1842, p. 13, pl. i, figs. 9—14 (egg-capsules); Leydig, 1849 b; Diesing (partim), 1850, p. 456; Grube, 1851, p. 110; Robin, 1865, p. 5 *et seq.* (embryology); Hertwig, 1877, p. 2 *et seq.* (embryology); Hoffmann, 1880 (embryology); Schneider, 1880, pp. 19 and 256 (embryology); Jijima, 1882, p. 12 (ovary and egg-strings); Bergh, 1884, p. 284, pls. xviii and xix (development); Filatow, 1898, p. 645 (embryology); Graf, 1899, p. 224

ct seq. (cytoanatomy, etc.); Brandes, 1899, p. 122 (copulation); Havet, 1899, p. 69 *et seq.* (nervous system); Brumpt, 1900 (cocoon); Sukatschoff, 1900, p. 618; Schuberg, 1904, p. 629 (with parasitic Nematodes).

Hirudo (*Erpobdella*) *vulgaris*, de Blainville (partim), 1827, p. 259; Gervais, 1836, p. 629, pl. ccxi, fig. 7.

La Nephelis vulgaire, Dugès, 1828, pp. 312 and 335, pl. ix, fig. 8 (egg-capsule).

Nephelis octoculata, Moquin-Tandon (partim), 1846, p. 302, pl. iii, figs. 1—11 (coloured), 12—33 (anatomy); Thompson, 1846, p. 389 (in Ireland); Apáthy, 1888 a, p. 154, etc., pl. viii, fig. 12 (head region); Apáthy, 1888 b; R. Blanchard, 1892 a, p. 171, fig 5, A and B (diagram of somite); R. Blanchard, 1893 a, p. 31 fig. 13, A and B (diagram of somite incorrectly copied from Blanchard, 1892, fig. 5); R. Blanchard, 1893 b, p. 8, figs. 3—5 (diagrams of annulation, etc.); R. Blanchard, 1893 e, p. 194 (identity with *N. sexoculata*, Schneider, established).

(Not *Nephelis tesselata* (?), Brightwell, 1842, p. 13, pl. i, figs. 15—17 = *Protoclepsis tessellata*.)

Hirudo octo-octulata, seu vulgaris, Dalyell, 1853, p. 14, pl. ii (coloured).

Nephelis sexoculata, Schneider, 1883, pl. iv, fig. 4.

Herpobdella octoculata, R. Blanchard, 1894 b, p. 52, figs. 15—17 (same as figs. 15—17 in Blanchard, 1893 b); Scharff, 1898, p. 194 (in Ireland); Evans, 1905, p. 215 (in Scotland).

Diagnosis. Body elongate, flattened, attenuated anteriorly, bluntly rounded posteriorly, of a uniform width posterior to the clitellum.

Colour deep brown, yellowish or reddish brown, paler ventrally, sometimes with blackish markings and a median dark stripe on the dorsal surface.

Somites I—IV uniannulate, V triannulate; the 18 somites VI—XXIII complete with five rings. Somites XXV—XXVII biannulate.

[Somite XXIV usually with four rings, occasionally with five owing to the subdivision of the last ring, similarly somite XXV is sometimes triannulate owing to the transverse subdivision of the second ring.] Eight eyes. The first and second pairs lie in a transverse curved line on somite II; not infrequently the second (and outer pair) lie between somites II and III or entirely in somite III. The third and fourth pairs are situated on the first ring of somite V, but may encroach to some extent upon the succeeding ring.

The male genital orifice lies between rings 36 and 37, that is, between the fourth and fifth rings of somite XI; the female orifice is four rings behind the male, between the third and fourth rings of somite XII.

The anus lies between the two rings of somite XXVI.

Length 30—50 mm.; width 2—5 mm.

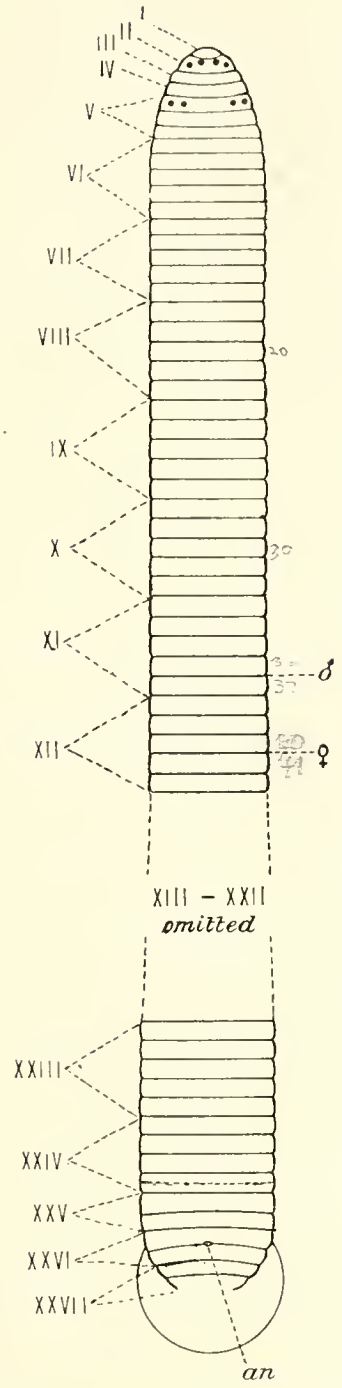

Fig. 14. *Herpobdella octoculata*. Diagram of dorsal surface. Somites numbered in Roman numerals. The eyes are indicated by black dots. *an*. Anus. (Adapted from R. Blanchard.)

Distribution, Food, etc. This species is widely distributed in Europe and very common in the British Islands, in running and stagnant water, upon the muddy bottom, beneath stones and among aquatic plants. It breeds from May to October and its dark brown, transparent egg-capsules are 4—6 mm. long and 2—4 mm. wide and attached at each extremity to the stalks of aquatic plants and other submerged foreign bodies.

This and the succeeding species devour small Oligochaetes such as *Tubifex*, Planarians (Moquin-Tandon) and probably a variety of other soft-bodied aquatic animals. Bristol (1898) fed the N. American *H. lateralis*, in confinement, upon "chopped fresh-water clams."

Herpobdella atomaria, *Carena*, 1820.

Plate XV, Fig. 45. Text Fig. 15.

Synonymy and Literature:

Hirudo atomaria, Carena, 1820, p. 295, pl. xii, fig. 16.
Nephelis atomaria, Moquin-Tandon, 1826, p. 128, pl. vi, fig. 6; R. Blanchard, 1892 a, p. 165, figs. 1—4; R. Blanchard, 1893 b, p. 4, figs. 1, 2.
Hirudo (*Erpobdella*) *atomaria*, de Blainville, 1827, p. 261, pl. xxxvi, fig. 5.
N. octoculata, var. *atomaria*, Moquin-Tandon, 1846, p. 304, pl. iii, fig. 7.
N. reticulata, Malm, 1860, pl. iii, fig. 7.
N. scripturata, Schneider, 1885, p. 129 (see R. Blanchard, 1893 d, p. 195).
N. crassipunctata, Schneider (see R. Blanchard, 1893 e, p. 197).
Herpobdella atomaria, R. Blanchard, 1894 b, p. 56, figs. 18—22.

Diagnosis. Body closely resembling *H. octoculata* in form, usually fulvous or greenish brown, paler and unicolorous ventrally.

Dorsal surface, except at the anterior extremity, with a series of reddish or yellowish white spots on every ring and generally with a black reticulated pattern. The first ring of each somite is rendered conspicuous by the accentuation of the yellowish white spots, which are often fused into a transverse band, and by the absence of black pigment.

Somites I—IV uniannulate, V and XXV usually biannulate but sometimes triannulate owing to the subdivision of the second ring, XXVI and XXVII biannulate; the 18 somites VI—XXIII complete with five rings. Somite XXIV usually quadri-, sometimes quinque-annulate owing to subdivision of the last ring.

The male genital orifice is situated between the fourth and fifth ring or upon the fifth ring of somite XI; the female orifice lies between the second and third ring or upon the third ring of somite XII. These

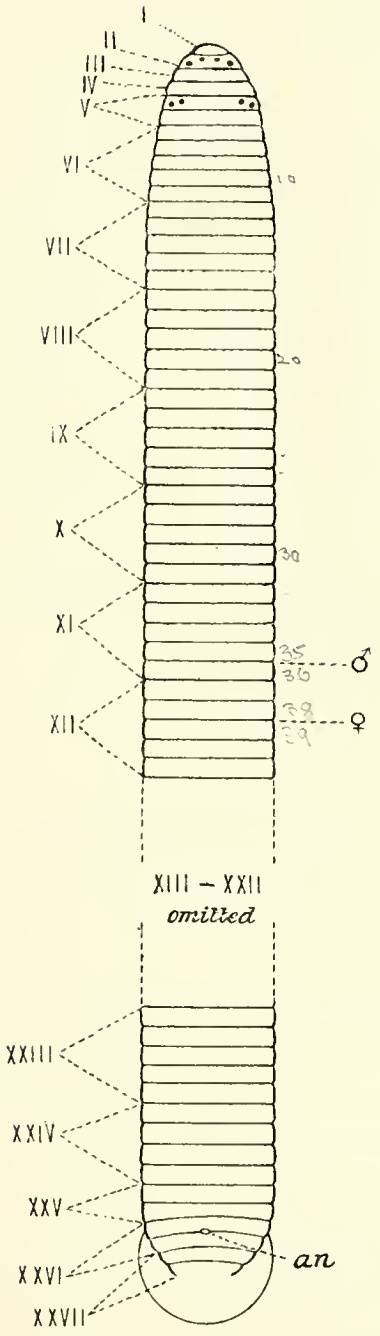

Fig. 15. *Herpobdella atomaria*. Diagram of dorsal surface. Somites numbered in Roman numerals. The eyes are indicated by black dots. *an.* Anus. (Adapted from R. Blanchard.)

orifices are usually separated by three rings; they are never separated by more than three and a half rings or by less than two and a half rings.

Length 50—70 mm.; width 4—6 mm.

Distribution, etc. This species, widely distributed in Europe, is found in the same situations as *H. octoculata* and subsists on the same food. It is probably of frequent occurrence in the British Islands. In the neighbourhood of Cambridge it is nearly as common as *H. octoculata*. The egg-capsules closely resemble those of the latter leech but according to L. Johansson (1909, p. 81, fig. 144) the openings at the ends of the capsule are closed by spherical plugs which project into the interior.

H. atomaria, it will be seen, differs from *H. octoculata* in the position of the genital openings, in its somewhat larger size, and generally in colouration and in small modifications in the external metamerism. Somite V, which is triannulate in the latter species, is usually biannulate in *H. atomaria*; colouration, which is variable in both species, is not always an infallible distinction between them and in doubtful cases we have to rely in the last resort upon the number of rings separating the genital apertures.

Series 2. **Epactodesminae.**

Complete somite with an intercalated ring.

Genus: **Trocheta**, *Dutrochet*, 1817.

Synonymy:

Trochetia, de Blainville (in Lamarck), 1818. *Hirudo* (*Trochetia*), de Blainville, 1827. *Geobdella*, de Blainville, 1828.

Amphibious Herpobdellidae with four pairs of eyes. Complete somite formed rarely of six rings all equal in size except the fourth (intercalated) ring, which is half the width of the others; and generally of a varying number of rings not exceeding eleven, due to the subdivision frequently of rings 5 *and* 6 *and occasionally also of rings* 1, 2 *and* 3; *the normal form consists of three equal large rings followed by five equal half rings.*

Trocheta subviridis, *Dutrochet*, 1817.

Plate XV, Figs. 46 and 47. Text Fig. 16 (p. 185).

Synonymy and Literature:

Trocheta subviridis, Dutrochet, 1817, p. 130; Moquin-Tandon, 1846, p. 309, pl. iv, figs. 1—5 (coloured), 6—21 (anatomy); Blanchard, 1892 d (occurrence in Liguria and descr.); Blanchard, 1893 b, No. 4, figs. 1—8 (fig. 6 is defective); Blanchard, 1893 c, p. 31, figs. 1—4 (annulation, etc.); Blanchard, 1894 b, p. 64, figs. 25—30 (diagrams of annulation, etc.), also p. 52, fig. 14 C, D, E (diagram of somite compared with *Herpobdella* and *Dina*); Diesing, 1850, p. 459; Johnston, 1865, p. 45; Gedge, 1869, p. 396.

Trochetia subviridis, de Blainville (in Lamarck), 1818, p. 292; Milne-Edwards (in Lamarck), 1838, p. 523; Bosc, 1819, p. 500; Schintz, 1822, p. 826; Cuvier, 1829, p. 215; Gray, 1850, p. 52; Gray, 1851, p. 429; Murie, 1865, p. 659; Lee, 1871, p. 21; Harting, 1877, p. 515 (occurrences in England, with references).

Nephelis gigas, Moquin-Tandon, 1826, p. 127, pl. vi, figs. 5 A—D.

Nephelis trochetia, Moquin-Tandon, 1826, p. 129.

Hirudo (*Geobdella*) *trochetii*, de Blainville, 1827, p. 246; Gervais, 1836, p. 628.

Erpobdella vulgaris, var. *gigas*, de Blainville, 1827, p. 564.

Hirudo (*Geobdella*) *viridis*, de Blainville, 1827, pp. 244 and 246; de Blainville, 1828, p. 559, pl. xxxiv, figs. 6, 6 a, 6 b.

Geobdella trochetii, de Blainville, 1828, p. 559, pl. xxxiv, fig. 3.

Trocheta cylindrica, Orley, 1886.

Nephelis trocheta, Apáthy, 1888 a, p. 154 *et seq.*, 1888 b.

Diagnosis. Body elongate, vermiform, more or less cylindrical anteriorly, posteriorly flattened, with keeled margins. Colour grayish green or reddish, paler ventrally; generally with two longitudinal, paramedian brown lines upon the dorsal surface.

Somites I—IV and XXVI—XXVII uniannulate, V and XXV biannulate; the 19 somites VI—XXIV complete and composed generally of three large rings followed by five small rings.

Clitellum more or less swollen and conspicuous, beginning with the third large ring of somite X and extending to and including the second large ring of somite XIII.

The male genital orifice is situated between somites XI and XII or upon, or immediately anterior to, the last short ring of somite XI. The female orifice lies immediately behind, immediately before, or upon, the short ring which lies posterior to the intercalated ring of somite XII. The eight eyes are disposed in two groups of four each, as in *Herpobdella*; the anterior group lie in ring 2 and the posterior group in ring 5. Anus large and prominent, between the two rings of somite XXV.

Length, at rest, 80—100 mm.; fully extended, 200—215 mm. Width, 7—15 mm.

Distribution, Food, etc. This species occurs in France, where it was first noticed by Dutrochet (1817, p. 130) near Chateau-Renaud (Indre et Loire); in Italy where Blanchard (1892 b) found it in great abundance in the Ligurian Apennines, and in Algeria (Moquin-Tandon, 1846, p. 310). It has not been recorded from Scotland or Ireland, and in England it has appeared at rare intervals, but on more than one occasion in considerable numbers. Harting (1877, pp. 515—523) has collected all available information relating to its occurrences, real or alleged, in this country up to the year in which he wrote.

The first reliable record of the appearance of Dutrochet's leech in England is given by Gray (1850, p. 52) who refers to a single example taken in Regent's Park, which was sent alive to the London Zoological Society's Gardens and subsequently added to the British Museum Collection and catalogued by Johnston (1865, p. 45).

Lee (1871, p. 21; cited by Harting, *loc. cit.*) found it on the Croydon Sewage-irrigation Farm at Beddington and noted its occurrence, upon hearsay evidence only, (1) on the Sewage Farm belonging to the same town, at Norwood, (2) in Hampshire, and again (3) in abundance, at Lindfield in Sussex.

Harting (*loc. cit.* p. 521) refers to a considerable correspondence relating to this species which appeared in the Natural History columns of *Land and Water*, in 1869, and elicited the fact that Mr Broadwood had noticed for many years previously examples of *T. subviridis* on the lawns and paths of his garden at Lyne, between Dorking and Horsham, in Surrey. I have had the opportunity of examining three specimens from this source, presented to the University Museum of Zoology at Cambridge by Mr M. R. Pryor, from whom I learn that this leech has been recorded again recently from the same locality. Mr Pryor informs me further that he has taken *T. subviridis* in the catch pits of drains in garden paths. He reports it from Elstree in Hertfordshire and the Cambridge Museum possesses an example taken by him at Capel, in Surrey, in 1891. In May, 1909, Dr F. W. Gamble was good enough to send me some examples of *T. subviridis* which had been found at the Withington Sewage Works, near Manchester, where these leeches frequent the sewage effluent channels and devour the earthworms which are washed out of the contact filtration beds. To the courtesy of Mr Hugh Stowell, Chief Inspector of the Mersey and Irwell Rivers Board and Mr C. H. Ball, Manager of the Withington Works, I am indebted for additional material and information. Its occurrence, except in the last instance, at no place very remote from London, its first discovery in Regent's Park and the fact that several leeches described

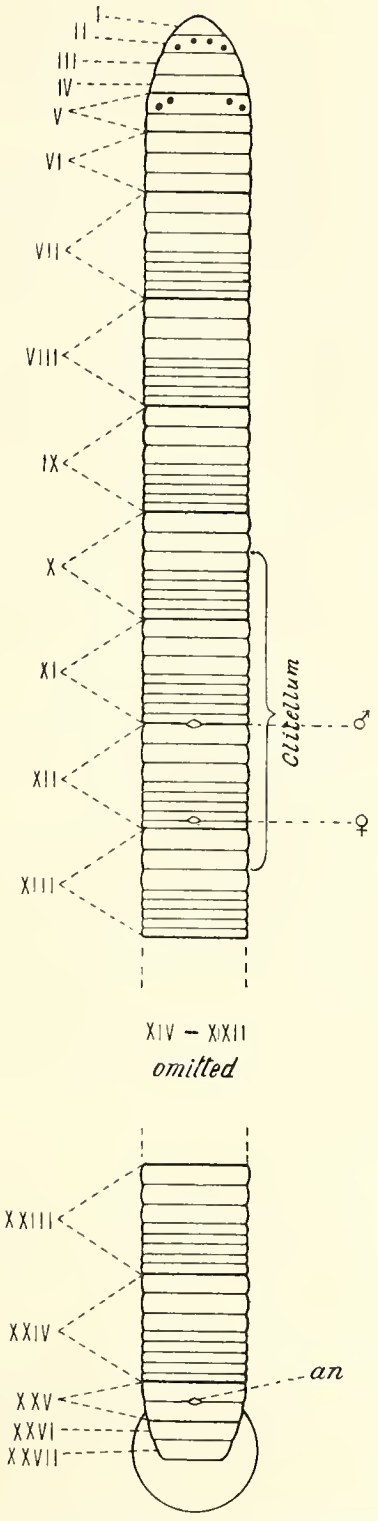

Fig. 16. *Trocheta subviridis.* Diagram of dorsal surface. Somites numbered in Roman numerals. The eyes are indicated by black dots. *an.* Anus. (Adapted from R. Blanchard.)

as *T. subviridis* have been reported from the Zoological Society's Gardens have led to the supposition that this species has been introduced together with some foreign animal, and that the latter locality has been its centre of distribution.

There is little, however, to substantiate this view. The geographical range of *T. subviridis* is, as we have seen, somewhat limited, on the other hand a number of closely allied forms occur in the Oriental Region for which it might be mistaken. To an Oriental species indeed (*Whitmania ferox*) Blanchard (1896, p. 322) has referred the leech described as *T. subviridis* by Muric (1865, p. 659), taken *post mortem* from the viscera of a Moluccan deer in the Zoological Society's possession. The identity again of the land leeches found in 1877 by Professor Garrod in the above Society's Gardens and referred to by Harting (*loc. cit.*) as probable examples of Dutrochet's leech is by no means beyond dispute.

The species in question is in fact rare but indigenous in this country, although the factors which determine its somewhat sporadic occurrences are obscure. It is probably not so uncommon as is generally supposed. Its superficial resemblance to an earthworm may not infrequently have caused it to be overlooked and our ignorance of this species, as in the case of several other English leeches, is undoubtedly largely due to the small amount of attention which British Hirudinea have hitherto received.

Trocheta subviridis is carnivorous, devouring piecemeal various species of earthworms and also (Blanchard, 1894 b, p. 64) the larvae of insects. It is amphibious, frequently leaving the water in order to pursue its prey in moist situations upon land. According to Moquin-Tandon (1845, p. 312) the egg-capsules, which are elliptical, flattened, dark brown and nearly opaque, are attached by their extremities, as in the case of *Herpobdella*, to some foreign body and attain a length of 9—14 mm. and a width of 6—8 mm.

LIST OF BRITISH HIRUDINEA.

Sub-order I. RYNCHOBDELLAE.

Family I. **ICHTHYOBDELLIDAE.**

Genus. Branchellion.

Species. **B. torpedinis.**

Genus. Trachelobdella.

Species. **T. lubrica.**

Genus. Piscicola.
Species. **P. geometra.**
Genus. Pontobdella.
Species. **P. muricata.**

Family II. **GLOSSOSIPHONIDAE.**

Genus. Protoclepsis.
Species. **P. tessellata.**
Genus. Hemiclepsis.
Species. **H. marginata.**
Genus. Glossosiphonia.
Species. **G. heteroclita.**
Species. **G. complanata.**
Genus. Helobdella.
Species. **H. stagnalis.**

Sub-order II. ARHYNCHOBDELLAE.

Family I. **GNATHOBDELLIDAE.**

Sub-family. Hirudininae.
Series 1. **Distichodonta.**
Genus. Haemopis.
Species. **H. sanguisuga.**
Series 2. **Monostichodonta.**
Genus. Hirudo.
Species. **H. medicinalis** (*extinct*).

Family II. **HERPOBDELLIDAE.**

Series 1. **Haplodesminae.**
Genus. Herpobdella.
Species. **H. octoculata.**
Species. **H. atomaria.**
Series 2. **Epactodesminae.**
Genus. Trocheta.
Species. **T. subviridis.**

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[1] N.B. I have followed Diesing (1850) in attributing the nomenclature in Lamarck's work (1818) to de Blainville. The latter writer (1827, p. 206) states that "M. de Blainville...a proposé les mêmes subdivisions que M. de Lamarck a adoptées de ses manuscrits," but there is no internal evidence of any further contribution.

We are expressly told by de Blainville that he suggested to Lamarck the names *Piscicola* and *Erpobdella* (1828, pp. 558 and 564).

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DESCRIPTION OF PLATES XIII—XV.

PLATE XIII.

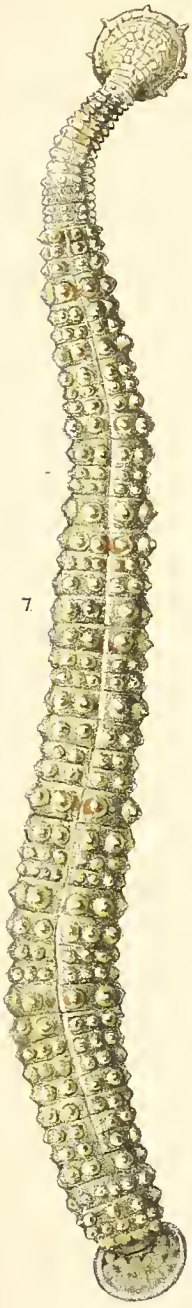
Fig. 1. *Branchellion torpedinis*, from an example in the Cambridge University Museum of Zoology. Dorsal aspect. ×1½.
Fig. 2. The same. Anterior extremity. Ventral aspect. ×3.
Fig. 3. The same. Interior of part of posterior sucker, much magnified.
Fig. 4. *Pisicola geometra*. Dorsal aspect. ×3.
Fig. 5. The same. Ventral aspect. Yellow example. ×3.
Fig. 6. The same. Egg capsule, *a*, natural size, *b*, magnified.
Fig. 7. *Pontobdella muricata*, nat. size.
Fig. 8. The same, at rest—a characteristic position, nat. size.
Fig. 9. The same. Yellow variety.
Fig. 10. The same. Egg capsules attached to a fragment of shell, nat. size.
Fig. 11. The same. Egg capsule, side view, much magnified.
Fig. 12. The same. Egg capsule, end view, much magnified.

PLATE XIV.

Fig. 13. *Helobdella stagnalis*. Dorsal aspect. ×4.
Fig. 14. The same. Fully extended, nat. size.
Fig. 15. The same. At rest, nat. size.
Fig. 16. The same. Ventral aspect. ×4.
Fig. 17. The same. Ventral aspect with adhering young, magnified.
Fig. 18. *Glossosiphonia heteroclita*. Dorsal aspect. ×4.
Fig. 19. The same. Ventral aspect with eggs and emerging embryos. ×4.
Fig. 20. The same. Small example, dorsal aspect, nat. size.
Fig. 21. The same. Ventral aspect with eggs, slightly magnified.
Fig. 22. *Glossosiphonia complanata*, var. A. Dorsal aspect. ×3.
Fig. 23. The same. Ventral aspect. ×3.
Fig. 24. The same, var. B. Dorsal aspect. ×3.
Fig. 25. The same. Position assumed when disturbed, nat. size.
Fig. 26. The same, var. C. Part of dorsal surface. ×3.
Fig. 27. The same. In outline, nat. size.
Fig. 28. *Hemiclepsis marginata*. Seen against an opaque background. Dorsal aspect. ×4.
Fig. 29. The same individual rather more contracted, seen as a transparency. Dorsal aspect. ×4.
Fig. 30. The same individual. Ventral aspect, seen as a transparency. ×4.
Fig. 31. The same. In outline, nat. size.
Fig. 32. The same, var. *flava*. Part of dorsal surface. ×4.

PLATE XV.

Fig. 33. *Protoclepsis tessellata*. Dorsal aspect, extended. ×4.
Fig. 34. The same. Ventral aspect, extended. ×4.
Fig. 35. The same. Contracted. ×4.
Fig. 36. *Hirudo medicinalis*, from an imported example, nat. size.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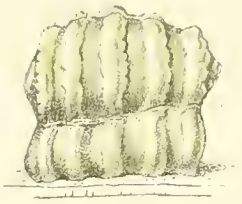
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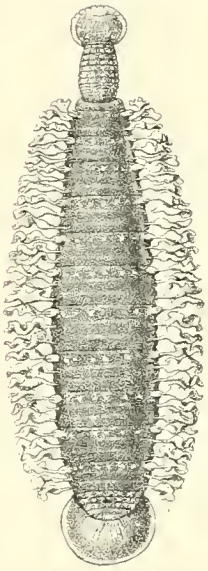
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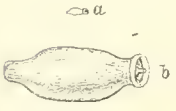

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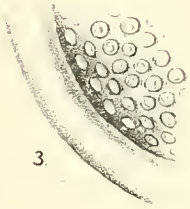
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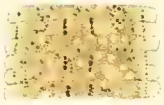
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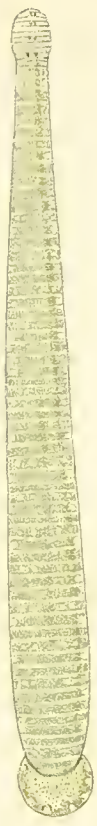

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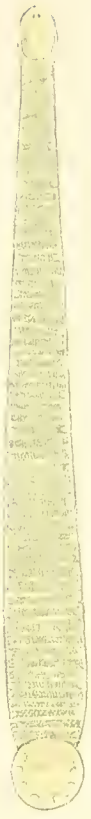

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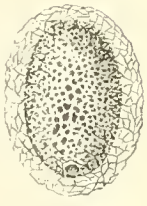

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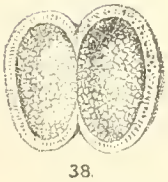

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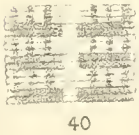

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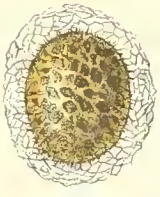

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Fig. 37. The same. Cocoon, nat. size. (Adapted from J. R. Johnson.)
Fig. 38. The same cocoon, cut open, nat. size. (After J. R. Johnson.)
Fig. 39. *Haemopis sanguisuga*, nat. size.
Fig. 40. The same. Portion of primitive dorsal pattern seen in a young individual. Magnified.
Fig. 41. The same. Cocoon, nat. size.
Fig. 42. *Herpobdella octoculata*. Small example, swimming, nat. size.
Fig. 43. The same. Egg capsule, nat. size.
Fig. 44. The same egg capsule, magnified.
Fig. 45. *Herpobdella atomaria*. Dorsal aspect. ×2.
Fig. 46. *Trocheta subviridis*. Dorsal aspect, nat. size.
Fig. 47. The same. Egg capsule. (After Moquin-Tandon.)

THE DEGENERATIVE APPEARANCES OBSERVED IN *PIROPLASMA CANIS* AND IN *TRYPANOSOMA BRUCEI* FOLLOWING UPON DRUG TREATMENT.

By GEORGE H. F. NUTTALL, F.R.S.

(2 Diagrams.)

In a lecture delivered recently in Cambridge, I had occasion to demonstrate the degenerative changes undergone by *P. canis* and *Tr. brucei* in the blood of animals which had been subjected to curative treatment with drugs. These changes have not as yet been depicted. They are, in my opinion, of more than passing interest, since they may help us to distinguish normal from abnormal parasites in untreated animals. Without doubt there occurs a certain death-rate amongst blood parasites under natural conditions; what this death-rate is we do not know, but appearances which cannot be regarded otherwise than as degenerative are not infrequently encountered. On the other hand, there is always a danger that false interpretations may be placed upon abnormal or degenerative forms, and of this I fear there is ample evidence in current literature. In publishing this note I merely desire to draw attention to what may prove to be a useful method of differentiating some of the normal from the abnormal appearances presented by Haematozoa.

I. *The degeneration observed in Piroplasma consequent upon Trypanblue treatment.*

In our papers on the successful drug treatment of canine and bovine piroplasmosis (*Parasitology*, vol. II. 1909, Nuttall and Hadwen, pp. 163, 190, 249, 265; Nuttall, pp. 418, 432) we described how *P. canis* and *P. bovis* degenerated under the influence of trypanblue, the appearances observed in both species of parasites being similar. To repeat,

the essential changes observed consist (*a*) in the disappearance of the typical intracorpuscular pyriforms, whilst (*b*) the surviving parasites appear rounded or irregular prior to their disappearance from the peripheral circulation. In stained blood films (Giemsa) the degenerating parasites often show a pale, ragged or irregular appearance, and masses of chromatin are frequently extruded. Viewed in fresh films, the parasites usually appear rounded. The changes undergone by *P. canis*, and this holds equally for *P. bovis*, consequent upon the treatment of an animal with trypanblue, are depicted in the accompanying diagram.

Diagram 1, Figs. 1—4, represent normal and common types of parasites occurring in the blood of a dog *before treatment*: a rounded form (O), a pair of pyriforms (PP), two pairs of pyriforms (PPPP) and a dividing form (D). Frequent reference has been made to these types

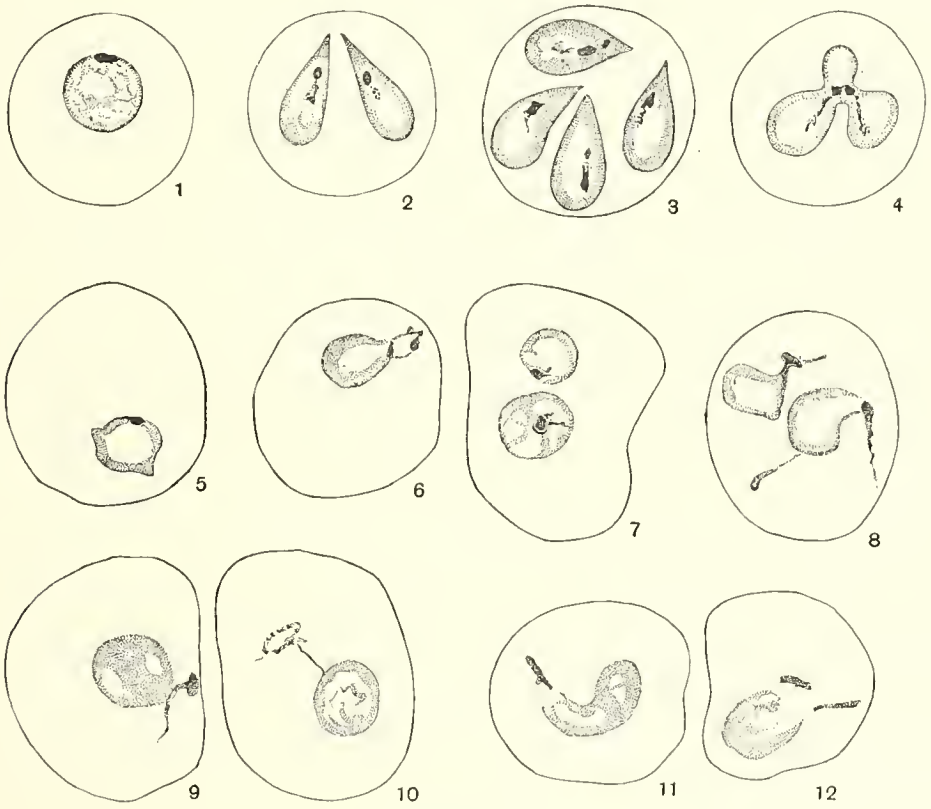

Diagram 1.

Showing the degeneration of *Piroplasma canis* consequent upon the treatment of a dog with trypanblue. Figs. 1—4, normal types of parasites (see text). Figs. 5—12, parasites from the same dog, 6 hours after the injection of trypanblue; parasites degenerating. After coloured figures, from Giemsa-stained blood films, drawn with a Winkel camera lucida and Zeiss 2 mm. objective with eyepiece 18 (× 3000).

of *Piroplasma* in the papers referred to above. The remaining figures (Figs. 5—12) show the appearances of the parasites in the same animal six hours after the injection of a curative dose of trypanblue. In Fig. 5 we have a common type, the parasites being irregular or rounded in form and shrunken. Pairs of pyriforms are no longer encountered or they are very scarce. Where two parasites occur in a corpuscle they appear rounded or irregular (Figs. 7, 8). In Fig. 8 the chromatin is drawn out like a flagellum to one side, reminding one of certain parasites which various authors have wrongly regarded as flagellate forms of *Piroplasma*. In most parasites the protoplasm stains faintly blue, and frequently, as is shown in the figures, the chromatin assumes fantastic shapes and is extruded. Figs. 6 and 7, 9 and 10, 11 and 12 represent pairs of corpuscles containing degenerating parasites and lying side by side in the blood-film from which the drawing was made.

In untreated dogs, it is rare to encounter parasites extruding their chromatin. We have noted this elsewhere (Nuttall and Graham-Smith, 1906, *Journ. of Hygiene*, VI. p. 597, Diagram 7; p. 598, Diagram 9, Figs. 21, 23).

II. *The degeneration observed in Trypanosoma brucei consequent upon Arsenophenylglycin treatment.*

The effect of arsenophenylglycin upon *Tr. brucei* in treated mice is very marked, and is recorded in the following tables. The dose of the drug administered subcutaneously was 1 c.c. of a 1:70 aqueous solution per 20 gramme mouse.

Mouse I.

Time	No. of tryps. per 1000 r.b.c.	% of tryps. showing: No granules	% of tryps. showing: Granules	% of tryps. containing: 1-2 granules	% of tryps. containing: 3-5 granules	% of tryps. containing: Many granules	% pale	% rounded or breaking up	% pointed	% blunt
Before treatment:										
1 hr.	12	95	5	4	0	1	0	0	92	8
After treatment:										
1 hr.	10	5	95	19	44	31	1	0	0	100
2 hrs.	8	0	100	4	22	69	1	4	0	96
3 ,,	0·6	0	100	2	16	62	11	9	0	91
5 ,,	0·2	—	—	—	—	—	—	—	—	—

The number of trypanosomes counted for every 1000 red blood corpuscles in a stained film of peripheral blood gives an adequate idea of the degree of infection both before and at various times after treatment.

Thus in Mouse I. there were 12 trypanosomes per 1000 r. b. c. present in the blood before treatment. Two hours after treatment the proportion was 8 : 1000, and after 5 hours only 0·2 : 1000. No parasites were subsequently discovered.

Whereas before treatment 95 % of the trypanosomes contained no dark red or purplish spherical granules in their blue-staining protoplasm, and 92 % showed blue-staining protoplasm protruding beyond the blepharoplast in the characteristic beak-like manner (Diagram 2, Fig. 1), already one hour after treatment only 5 % of the parasites were free from granules and none showed the beak-like process posteriorly. After two hours all of the trypanosomes showed granules (Diagram 2, Figs. 2, 3). As will be seen from the table, the proportion of trypanosomes containing 1—2 or 3—5 granules was very high one hour after treatment, but their number became less subsequently. On the other hand, the proportion of trypanosomes containing many granules increased after the second hour and decreased slightly after the third hour. After three hours no less than 12 % of the trypanosomes stained faintly (Fig. 4), some taking on a violet tint, whilst 9 % had become rounded or were breaking up. After five hours (Figs. 5—7) only a few pale-staining parasites, fragments of parasites, or loose flagella could be found. During the first three hours after the administration of the drug 21 to 29 % of the trypanosomes were found in various stages of longitudinal division as evidenced by the existence of two blepharoplasts and partial separation into two of the flagellar filament. These dividing forms, however, showed the same appearances of degeneration as those described above for the ordinary single parasites.

A second experiment was now carried out as follows. Two mice, each weighing about twenty grammes were inoculated at 10 a.m. on 15. II. 1910 with a small amount of blood obtained from the tail of a mouse infected with *Tr. brucei*. A very few trypanosomes were detected in the blood of both mice on 17. II. Counts of the number of trypanosomes in the blood of these mice were made commencing on 18. II., blood-films being prepared at intervals as stated in the protocols. One mouse (II.) was treated with arsenophenylglycin while the other (III.) was left untreated. There were 10 trypanosomes per 1000 r.b.c. present in the blood of the mouse (II.) immediately before treatment; blood-films

were prepared from this mouse every half-hour and up to 7 hours after the injection of the drug. Mouse III. served as a control; blood-films were prepared at longer intervals in this case. All of the blood-films were stained intensely by Giemsa, *i.e.* for 2 hours at 25° C., and only the central part of the films was examined for the purpose of making the enumerations.

After $5\frac{1}{2}$—6 hours only a few parasites could be detected; they were granular. After $6\frac{1}{2}$ hours two parasites were detected, the one

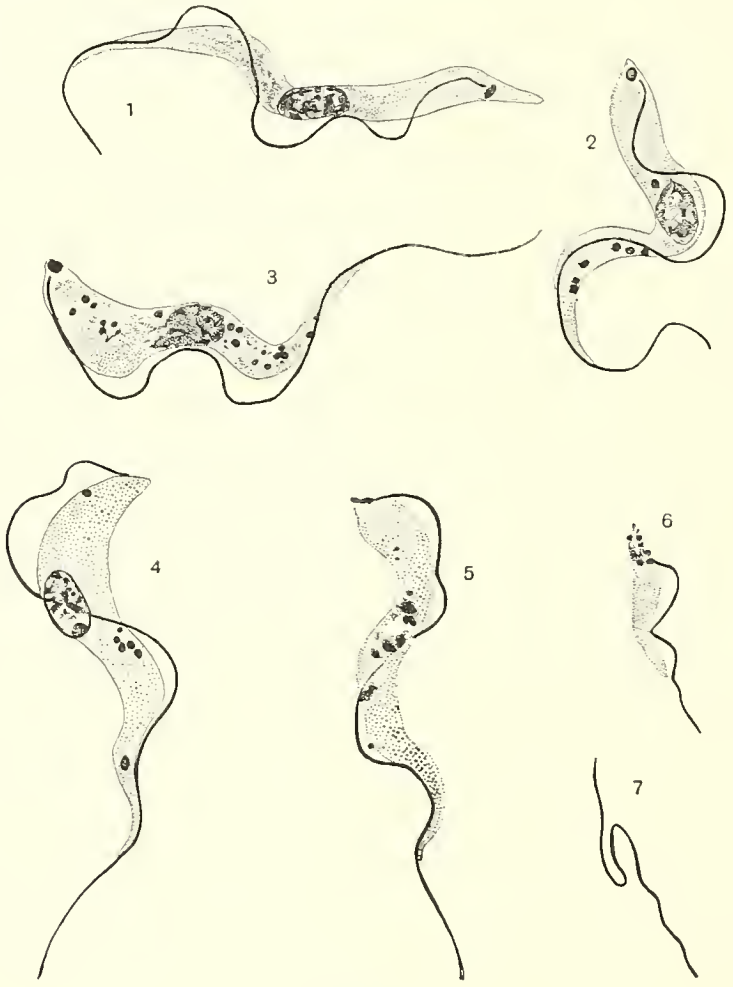

Diagram 2.

Showing the degeneration of *Trypanosoma brucei* consequent upon treatment of a mouse with arsenophenylglycin. Fig. 1, normal trypanosome. Figs. 2—3, parasites 2 hours after treatment. Fig. 4, a parasite 3 hours after treatment. Figs. 5—7, parasites and detritus 5 hours after treatment. After coloured figures from Giemsa-stained blood-films, drawn in the same manner as the figures in Diagram 1.

granular, the other rounded and breaking up. After 7 hours only one parasite was found per 30,000 r.b.c. enumerated; this parasite was pale, rounded and breaking up.

Treated Mouse II.

Time	No. of tryps. per 1000 r.b.c.	% of tryps. showing: No granules	% of tryps. showing: Granules	% of tryps. containing: 1-2 granules	% of tryps. containing: 3-5 granules	% of tryps. containing: Many granules	% pale	% rounded or breaking up	% pointed behind	% rounded behind	Parallel observations on the living parasites
Before treatment, 18.II.'10:											
10.40 a.m.	10	86	14 f *	8	4	2	0	0	100	0	—
After treatment:											
½ hr.	10	28	72 ffc	10	30	32	2	0	18	82	Tryps. more active than normal.
1 ,,	7	2	98 fc	4	40	52	0	2	16	82	Still more active.
1½ hrs.	6·5	0	100 fcc	0	10	90	14	6	2	92	Less active, appear granular.
2 ,,	5	6	94 ffc	8	26	60	48	6	0	94	Ditto.
2½ ,,	4	2	98 fffc	2	18	78	94	18	0	82	Ditto, but more granular.
3 ,,	4	42	58 fffc	28	16	14	100	6	0	94	Ditto.
3½ ,,	2·5	44	56 fffc	22	24	10	100	14	0	86	Ditto.
4 ,,	1·0	0	100	0	0	100	100	12	0	88	Still less active, granular.
4½ ,,	0·16	0	100	0	4	96	100	·	0	·	Ditto.
5 ,,	0·14	0	100	0	16	84	100	16	0	·	Much degenerated, very granular, flagellum moving slowly.
5½ ,,	0·13	0	100	·	·	·	·	·	·	·	Ditto (only 2 tryps. seen).
6 ,,	0·06	·	·	·	·	·	·	·	·	·	Ditto (ditto).
6½ ,,	0·06	·	·	·	·	·	·	·	·	·	No tryps. found.
7 ,,	0·03	·	·	·	·	·	·	·	·	·	Ditto.

* f = granules all fine, fc = granules growing coarser, ffc = granules mostly fine, fcc = granules mostly coarse, fffc = granules mostly very fine.

Owing possibly to the intense staining process to which the blood-films were exposed a somewhat lower percentage (86 %) of the trypanosomes was found to be free from granules in Mouse II. before treatment was commenced. Following upon treatment the percentage of granular parasites rose rapidly, but after 3—3½ hours many pale trypanosomes were noted which contained no granules. After 1½ hours no trypanosomes with pointed posterior extremities were enumerated. All of the parasites appeared pale after 3 hours, and after 2½ hours a considerable

number appeared rounded or breaking up. As in the previous experiment the number of trypanosomes had begun to decrease already 1 hour after the drug was injected. It will be noted that the granules grew coarser up to $1\frac{1}{2}$ hours after treatment and subsequently appeared to grow finer. In a number of cases the coarser granules took on almost a blackish tint and were arranged in rows in the degenerating trypanosomes.

The trypanosomes, viewed under the microscope in fresh blood, showed increasingly active movement up to 1 hour after the drug was injected, but after $1\frac{1}{2}$ hours they appeared granular and their movements grew slower. After 5 hours only the flagellum was seen to move slowly. Whereas there were 10 trypanosomes present per 1000 r.b.c. before treatment, there was but 1 trypanosome per 30,000 r.b.c. present 7 hours after treatment.

In the case of the untreated mouse it is interesting to note that the percentage of granular typanosomes increased rapidly on the day on which the animal died. The proportion of trypanosomes containing many granules also increased as the time of death approached. Four hours before the mouse died 12 % of the trypanosomes no longer showed pointed extremities. It appears therefore as if similar degenerative appearances occur in trypanosomes both in treated and untreated animals, in the latter case only on the approach of the host's death.

Untreated Mouse (Control to Mouse II.).

Time	No. of tryps. per 1000 r.b.c.	% of tryps. showing		% of tryps. containing			% pale	% rounded or breaking up	% pointed behind	% rounded behind
		No granules	Granules	1-2 granules	3-5 granules	Many granules				
18. II. '10										
10.40 a.m.	2	74	26	10	6	10	0	0	100	0
2.30 p.m.	11	90	10	2	4	4	0	0	94	6
4.30 p.m.	10	90	10	10	0	0	0	0	98	2
6 p.m.	10	98	2	2	0	0	0	0	98	2
19. II. '10										
9.45 a.m.	48	22	78	24	28	26	0	0	100	0
1 p.m.	24	2	98	0	28	70	0	0	88	12
5 p.m.	Mouse died.									

The observations above recorded indicate that the following changes take place in the trypanosomes consequent upon the drug treatment :— They retract their "beaks," and deep-staining reddish or purplish granules

appear in the protoplasm especially toward the flagellar end. The number and size of these granules increase rapidly, after which they disappear with the progressive dissolution of the parasite. After a time only nuclear detritus, portions of the ectoplasm and the flagellar filaments remain, and finally all traces of the parasites disappear. The red- or purple-staining rounded granules doubtless owe their origin to the breaking up of the nuclear chromatin, but the large number of granules observed in some cases suggests that they may also have some other origin. Similar changes occur in trypanosomes in dying animals which have been left untreated. Future investigations will no doubt determine more regarding the origin and nature of these granules which react to chromatin stains[1].

In conclusion, I desire especially to thank Geheimrath Ehrlich for placing a supply of arsenophenylglycin[2] at my disposal.

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[1] With regard to these granules we may note the following: Woodcock (1906, p. 228, Fig. 21) gives a brief description of the involution forms in which chromatolysis vacuolisation and change of form occur. In chromatolysis, according to Woodcock, the chromatic constituents of the nucleus pass out into the protoplasm or else direct fragmentation of the nucleus occurs.

Swellengrebel (1909, p. 87 *et seq.*) evidently refers to similar extranuclear granules occurring in *T. gambiense* and which he observed in the later stages of infection. He does not regard them as consisting of chromatin but of a substance allied to volutin. They stain red by Giemsa and are scarce in the early period of infection. Some of his figures much resemble what I have seen.

[2] See in this connection Roehl, W. (11. iii. 1909). Heilversuche mit Arsenophenylglycin bei Trypanosomiasis, *Zeitschr. f. Immunitätsforsch. u. exper. Therapie*, i. pp. 633—649.

A NEW FLAGELLATE (*MACROSTOMA MESNILI* N. Sp.) FROM THE HUMAN INTESTINE WITH SOME REMARKS ON THE SUPPOSED CYSTS OF *TRICHOMONAS*.

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(Plate XVI, and 2 Text Figures.)

The flagellate to be described in this paper was found in the faeces of a native of the Bahamas who was admitted to the Seamen's Hospital, Albert Dock, in October, 1909. In the course of the examination of the perfectly fresh material it was noticed that numerous flagellates were swimming about. They were taken to be *Trichomonas* but on closer examination they were seen to differ markedly from these. In the character of their movements there was little to distinguish them from *Trichomonas* except that the undulating membrane so characteristic of this genus appeared to be absent. The body is pear shaped and there are three long flagella directed forwards from the blunt end of the body. By the lashing of these three flagella the animal is drawn along with the blunt end in advance and at the same time revolves upon its longitudinal axis. A very characteristic feature is the presence of a very large cytostome extending from the base of the three flagella towards the posterior end of the body for about one half to two thirds of its length. The movements of the animals were at first so violent that great difficulty was experienced in making out the details of the anatomy, but as the faeces cooled their activities began to abate. It was then seen that the flagella was as long or longer than the body and that the large cytostome was arranged in a slightly spiral manner: that is to say it did not run directly parallel to the longitudinal axis of the body. The edges of the cytostome were produced into two lips which were very well shown when the animal was

viewed from the blunt end (Text Figures 1 and 2). Within the cytostome could be made out a flagellum or membrane displaying a constant undulatory movement. It was continued for the whole length of the cytostome from the insertion of the three flagella to the posterior end.

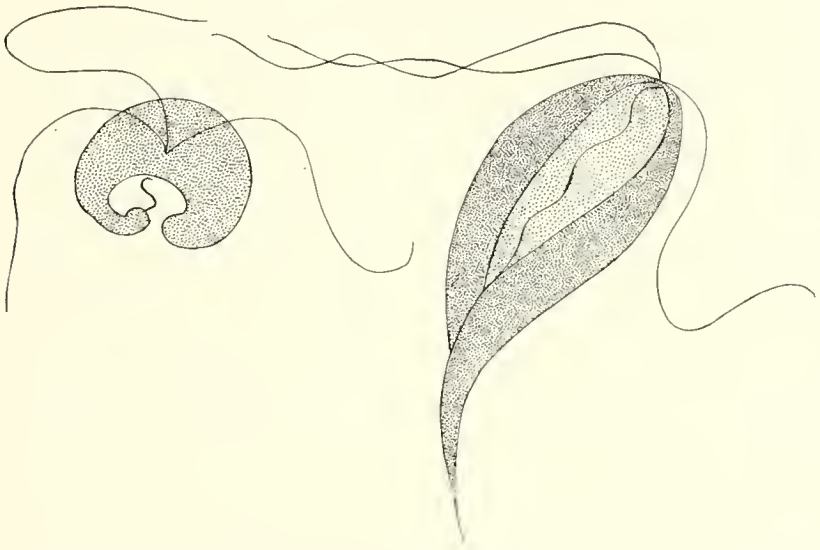

Fig. 1. Living flagellate viewed towards the blunt end. The lips of the large cytostome with the undulating membrane inside are well shown.
Fig. 2. Appearance of living animal.

It was very difficult to obtain a clear view of this structure so that some doubt exists as to whether this was an undulating membrane or a flagellum lying in the groove of the cytostome. If it had been a free flagellum it might have been expected that occasionally it would come out of the groove and give the flagellate the appearance of having four flagella. This was never found to occur nor did any of the appearances seen in the stained specimens lend support to this view. It seems safe therefore to conclude that the large cytostome had within it an undulating membrane. The general characters of the flagellate can be seen from Text Figures 1 and 2, which were drawn from life. The largest forms seen were about 15 μ long and 7 μ broad. Very much smaller forms were also met with and some not more than 3 or 4 μ across the longest diameter. The smaller forms were at first considered to be distinct organisms but from the stained specimens this was found not to be the case.

Fixing and Staining Methods.

The fixing and staining of these organisms and indeed of all intestinal Protozoa is best done by the method first advocated by Schaudinn. By this method or slight modification thereof it is possible to obtain beautiful preparations of the intestinal Protozoa with all the details of their anatomy preserved. If one attempts to make preparations by spreading faeces on a slide, allowing it to dry and then staining by some modification of the Romanowsky method, the results are most unsatisfactory and indeed the Protozoa are so broken up and disturbed in the drying process that they can hardly be recognised. In order to prepare specimens by the more rational method a small quantity of the faeces is spread out on a cover glass by means of a platinum loop or needle and without allowing it to dry it is dropped film side down on to the surface of a fixing fluid consisting of two parts of saturated aqueous sublimate and one part of absolute alcohol slightly acidified with acetic acid. This fixing fluid acts more quickly and better if warmed to the temperature of the body. Fixing is complete in a few minutes. The films are then carefully washed in weak spirit and finally in weak spirit to which a few drops of iodine solution have been added in order to completely remove the sublimate. The films are then placed in a 4 % solution of iron alum for several hours. They are then rinsed quickly for 2 or 3 seconds in distilled water to remove the excess of iron alum and immersed in Heidenhain haematoxylin (Haematoxylin crystals 1 gram dissolved in 10 c.c. absolute alcohol made up to 100 c.c. with distilled water. After ripening for a fortnight add 100 c.c. distilled water). In this stain the films are allowed to remain about 4—6 hours or longer. They are then quite black and after washing in distilled water are differentiated in a weaker solution (1 %) of iron alum. The progress of the differentiation must be watched by taking the films out of the solution from time to time, placing them on a slide with film side up and examining with the $\frac{1}{2}$ and $\frac{1}{6}$ inch objectives. When the nuclear structure and other details are clearly seen the film must be removed from the iron alum solution and washed well in water. The films are taken up through grades of alcohol to absolute alcohol, cleared in xylol and mounted in Canada balsam. It is most essential that the films be not allowed to dry at any point in this process. Prepared by this method or some slight modifications of it nearly all the details of structure of the intestinal Protozoa are very clearly brought out.

Morphology of the Parasite as observed in Stained Preparations.

In the stained films the flagellate now under consideration shows the same general characters as in the fresh specimens. In addition other details can be noted. At the anterior end of the body and a little to one side is a spherical vesicular nucleus. There is a definite nuclear membrane within which is an irregular network. One or more chromatin clumps are present and these are either situated at the centre of the nucleus or on the surface of the nuclear membrane. Near the nucleus is the insertion of the three flagella. These arise from a deeply staining chromatin granule. In some cases there appear to be two such granules from one of which arise two of the flagella, while from the other the remaining flagellum and possibly the marginal flagellum of the undulating membrane takes origin. However the exact details of the connection of this marginal flagellum with the chromatin granules could not be very clearly seen.

The protoplasm of the body is much vacuolated. Some of the vacuoles are food vacuoles and contain bacteria and other particles. The large cytostome with its two lips is very evident. The lips are most prominent at the middle of the cytostome and that of one side may overlap that of the other. The undulating membrane within the cytostome is visible as a wavy line. Exactly where this line takes origin on the line of insertion of the undulating membrane was not very clear. The position of this line within the cytostome and the fact that it was never seen outside it seems to indicate an undulating membrane rather than a flagellum. The posterior end of the animal is most frequently drawn out to a fine point. In other cases it is blunt while in some of the smallest forms seen the body is spherical. In these small forms the cytostome could not be seen but the three anterior flagella were quite visible. Division forms were not seen unless those with the two chromatin granules near the nucleus are interpreted as early division forms.

Encysted forms were encountered. These are oval bodies measuring $7\,\mu$ by $5{\cdot}5\,\mu$. Within these cysts the characteristic nucleus, the large cytostome and some other details could be seen. It was impossible to decide whether these cysts were reproductive or merely protective.

In addition to these undoubted cysts there occurred other bodies which appear to be derived from the flagellate. They are very similar

to the encysted forms of *Trichomonas* described by Ucke, Bohne and Prowazek and in greater detail by Bensen. In the present instance a most careful search failed to reveal any *Trichomonas*. However intermediate forms such as are shown in Plate XVI, Fig. 9 were frequently encountered so that it appears most probable that these peculiar bodies are derived from the flagellates. They occur in an almost endless variety, only a few of which are shown in Plate XVI, Figs. 9—13. Many of them are undoubtedly degenerate forms and all stages up to complete disintegration were met with. It seems most probable that these bodies are products of an abnormal development ultimately terminating in death. During this abnormal development vacuolations, changes in shape, segmentations of the body and various nuclear divisions of a peculiar type take place. Bodies with many nuclei may be formed but these all have the appearance of being abnormal. In the light of the fact that undoubted cysts (Plate XVI, Figs. 7 and 8) occur it seems impossible to arrive at any other conclusion.

The supposed Cysts of Trichomonas.

Bodies similar to these are constantly to be met with in human faeces. They may occur together with *Trichomonas*, and it is this association which has led Bohne and Prowazek and more recently Bensen to describe them as developmental cysts of these flagellates. Within these cysts a most complicated nuclear reduction and autogamy are described by these observers. These bodies may occur in association with *Trichomonas* but sometimes they are present in enormous numbers when no *Trichomonas* can be found, while at other times they bear no proportion to the number of *Trichomonas* present. If faeces containing these so-called cysts be kept either at laboratory temperature or at that of the body complete degeneration takes place within a few days. It has never been found possible to produce any development of these cysts outside the body on the warm stage as can be done with the cysts of *Entamoeba coli*. Further the origin of such a cyst from a living *Trichomonas* has never been followed though many attempts have been made to do so. It seems impossible to associate these structures with the flagellates unless as abnormal and degenerate forms. It is possible that such bodies can arise in another manner. Dobell has suggested that similar bodies, described by Prowazek as flagellate cysts from the intestine of lizards, are merely yeast cells and he has also thrown some

doubt upon the so-called *Trichomonas* cysts of Bohne and Prowazek mentioned above. These so-called cysts have been frequently encountered in the faeces of man and other animals, but it is interesting to note that in a previous work by the present writer on the *Trichomonas* of the mouse, though these flagellates often occurred in enormous numbers, no such cysts were met with.

The flagellate described in this paper shows undoubted affinities with *Trichomonas* and *Trichomastix*. Quite recently, under the name of *Macrostoma caulleryi*, Alexeieff has described a similar flagellate from the intestine of tadpoles. The shape of the body, the three anterior flagella and the large cytostome are features common to both forms. Alexeieff does not describe an undulating membrane within the cytostome as in the human form. It is just possible that this has been overlooked so that the new genus *Macrostoma* created by Alexeieff for the tadpole flagellate would also include the human form. For the human form the name *Macrostoma mesnili* may be suggested.

The presence of this flagellate in the intestine was productive of no ill effects, its presence being noted only in the routine examination of the faeces.

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PLATE XVI.

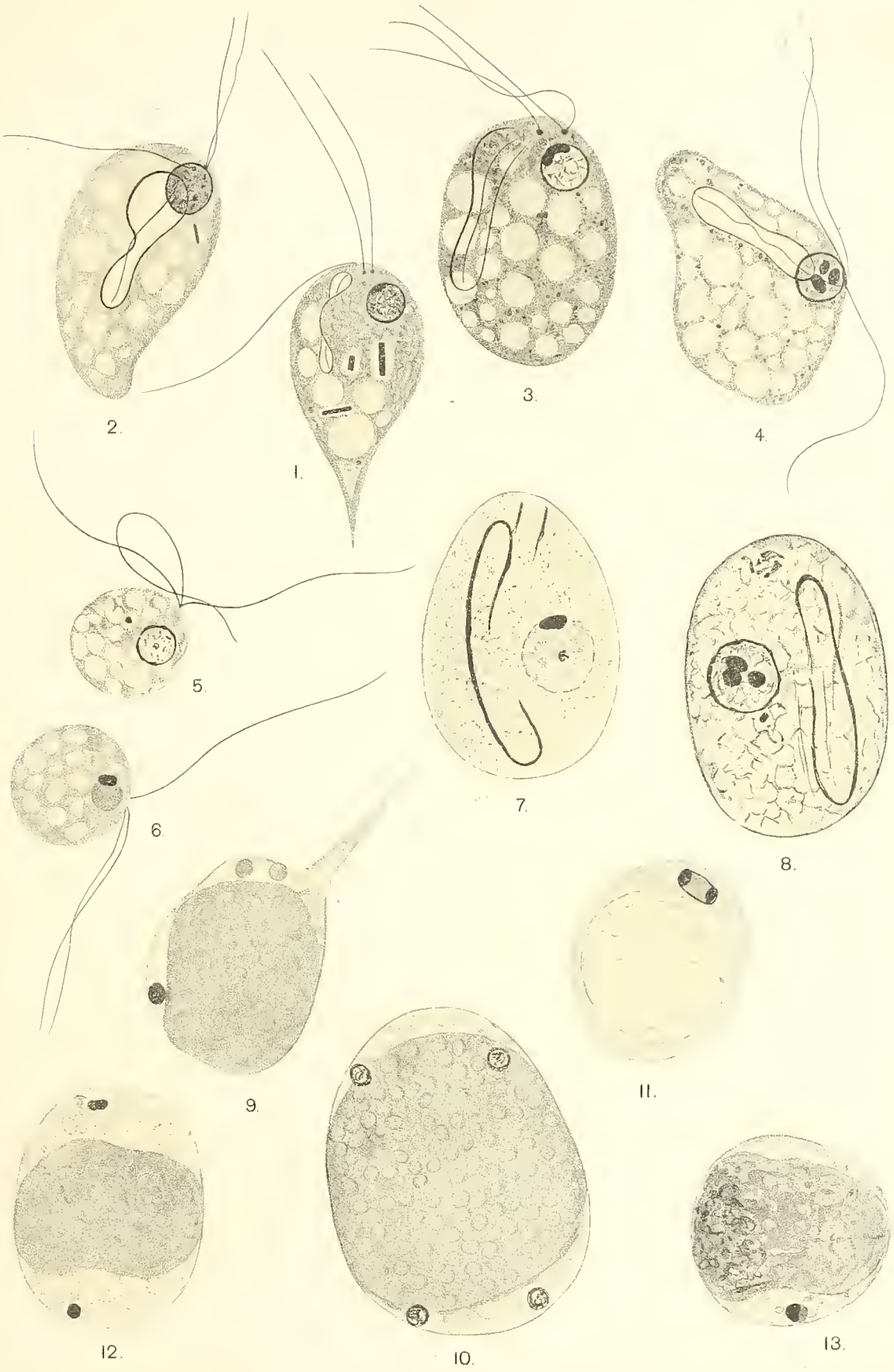
All the figures were drawn from stained specimens.

Figs. 1—4. Four flagellates showing well the different points of the large forms. The size of these is about $14\,\mu \times 7\,\mu$.

Figs. 5 and 6. Two of the smallest forms, not more than $3\,\mu$ in diameter. Note the absence of the cytostome which was possibly not visible on account of the small size.

Figs. 7 and 8. Encysted forms. The characteristic nucleus and the large cytostome are still visible within the cyst. $7\,\mu \times 5{\cdot}5\,\mu$.

Figs. 9—13. Bodies which are probably degenerative or abnormal developmental forms of the flagellate. Similar bodies have been described as encysted stages of *Trichomonas*. Fig. 9 shows the tail of the flagellate still present. All contain a large vacuole. The nucleus multiplies in a curious manner. These bodies quickly degenerate and break up when they pass out of the intestine.



E Wilson, Cambridge

NOTE ON THE SO-CALLED MUSCULATURE OF *TAENIA ELLIPTICA*.

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Plate XVII.

The internal organisation of the Cestoda is described as follows by Sedgwick (1898, p. 246):

"Beneath the cuticle-like outer membrane is a layer of spindle-shaped cells lying at right angles to the surface; their external ends abut upon the cuticle, and their inner ends are prolonged as fibres into the parenchyma. Beneath this layer there is a delicate superficial layer of longitudinal muscular fibres, and next a parenchyma of connective tissue in which strongly-developed bundles of longitudinal muscular fibres, as well as an inner layer of circular muscles, are embedded; both these muscular layers are traversed, principally at the sides of the body, by groups of dorso-ventral muscular fibres."

In *Taenia elliptica* a somewhat different arrangement is found which, so far as I know, has not been described, except, in part, for *T. lineata*, and which is, I think, worthy of record. Moreover, incidentally, a histological examination of this species throws some light on the relationship of contractile fibres to connective tissue cells which is not without interest.

Some of the proglottides of my specimens of *T. elliptica* were preserved in osmic acid, others in a mixture of corrosive sublimate and acetic acid. When hardened the osmic specimens were found to be flattened and elongated, while the others were shortened and expanded dorso-ventrally. Thus the behaviour of the various tissues under such different circumstances was to be seen and, owing to the different

effect of these two fixing agents on the tissues, light was thrown on the relationship of the various structural elements of the body and on their nature.

The anatomical arrangement of the structural tissues is as follows: A thickened structureless cuticle overlies a delicate basement membrane which surrounds the body. Within is a single layer of more or less elongated cells, lying perpendicular to the surface. These cells are connected with the basement membrane by branched processes, superficially, and by similar extensions internally they are directly continuous with the connective tissue parenchyma of the body and with certain dorso-ventral fibres described below. Between this layer of cells and the basement membrane, grouped in very small bundles lying between the superficial branched processes of these cells, is a delicate layer of longitudinal contractile fibres, called by me superficial longitudinal fibres. This layer is identical with a layer described by Hamann for *T. lineata* which he calls sub-cuticular muscles (1885, p. 718).

Below the outer layer of elongated cells and connected with them is the connective tissue parenchyma of the body; branched reticulated nucleated cells, in the meshes of which lie the various organs of the body. A central portion is however to some extent cut off from the external portion of the body by a layer of circular contractile fibres, thus dividing the body into three roughly equal sections, a dorsal, a central and a ventral section (Plate XVII, Fig. 1). Close outside this layer of circular fibres are a series of bundles of longitudinal contractile fibres forming a discontinuous layer of considerable bulk which must obviously exercise much power on the shape of the plastic proglottis and control the creeping movements of the free proglottides. Finally, throughout the body a great number of isolated dorso-ventral contractile fibres stretch from top to bottom of the body, and, branching superficially first into two and then into four tendrils, are connected with the internal processes of the outer layer of elongated cells. It is to be noted that these dorso-ventral fibres run through the bands of circular fibres, or even through a bundle of longitudinal fibres (Fig. 4) and are entirely independent of any other tissue or obstacle in their path.

The superficial layer of elongated cells which one is at first sight tempted to regard as epithelial elements is surely not such. Their superficial as well as their internal branching processes, the layer of longitudinal contractile fibres outside them (*s.l.f.*), their intimate connection with the connective tissue parenchyma, the character of their nuclei and their contractile power, are all indications that they are

nothing else than a concentrated layer of these connective tissue cells. That they are contractile is clearly shown, for, when the proglottis is contracted in length and swollen out dorso-ventrally, these cells are much elongated and are more crowded together (Fig. 2), while, on the other hand, when the proglottis is lengthened and flattened they are shortened and are not crowded together (Figs. 3 and 5). They no doubt serve the purpose of a protective layer, but they also serve as a means for extending the area over which any one of the dorso-ventral fibres exerts influence. One of these fibres, splitting up into two branches and then into four tendrils, is attached to four of these cells, whose superficial processes connect them with many points on the basement membrane and cause any contraction of that fibre to influence a considerable area of the surface of the body, a very important function.

The network of connective tissue parenchyma and its connection with the superficial layer of cells is well shown in Fig. 2. The meshes of the network are wide, and the anastomosing protoplasmic processes which form it are very delicate and prolonged. It is interesting to observe that several of these cells, lying close below the superficial layer, have long processes projecting between the cells of the latter.

Regarding the contractile fibres. There are four separate and independent systems which regulate the shape of the body or effect its movements, two distinct longitudinal, one circular and one dorso-ventral system.

These contractile fibres have usually been designated muscular, but the evidence I have to offer will I think demonstrate this is certainly not the case for all and probably not the case for any of them in *T. elliptica.*

In the specimens I obtained those preserved in osmic acid (Figs. 1, 3, 4 and 5) were elongated and contracted dorso-ventrally. The longitudinal fibres were thus stretched, while the circular and dorso-ventral fibres were contracted. In Figs. 1, 3 and 4 all these fibres are well shown, the small bundles of superficial and large bundles of deeper lying longitudinal fibres are seen in section, while the dorso-ventral and circular muscles are seen contracted. In all cases the fibres are sharply marked and are distinctly different in density from the processes of the connective tissue cells of the parenchyma.

On the other hand specimens preserved in corrosive sublimate and acetic acid (Fig. 2) were contracted longitudinally and distended dorso-ventrally. In these specimens the dorso-ventral and circular muscles are

stretched and appear as delicate gossamer fibres hardly distinguishable from the connective tissue and apparently of the same consistency. They are markedly different from the osmic specimens, being swollen and indistinct instead of wiry and of sharp contour. The same change, only still more marked, is seen in both groups of the longitudinal fibres; here there is no indication of separate fibres, they are swollen into a mass which is but faintly indicated, by shading, in my drawing.

This swelling under the influence of acetic acid is an attribute of white fibres and, when it is remarked there is no indication of any muscular structure in any of the fibres, it becomes exceedingly probable they are white fibres and not muscle fibres at all.

But there is another point; the dorso-ventral and circular fibres are each connected at one point, in the central section of the body, that is to say about the middle of their length, with a mass of protoplasm surrounding a large nucleus. The protoplasm is attached to the fibre but not enclosed in a sheath, as some observers have stated (*see* Pintner, 1881, p. 163), and this is to be seen in specimens preserved both in osmic acid and in corrosive sublimate and acetic acid. It is clearly shown in Figs. 3 and 4; in some cases the protoplasm surrounding the nucleus is attached to the fibre at two separate points (Fig. 3), in other cases it appears to lie flat on the fibre, but in all cases the fibre is quite sharply marked off from the nuclear protoplasm. A similar condition is seen in Fig. 2, but here, under the influence of acetic acid, the fibre itself is swollen and merges somewhat into the nuclear protoplasm.

The continuity of these contractile fibres with the cells from which it appears obvious they were derived, is an important matter in view of what I have to say below.

Finally in Figs. 3 and 4, the dorso-ventral and circular fibres, which are contracted, are seen to be spirally contracted. This is a very remarkable condition and so far as I know it has not been described in any cestode. Wavy contractions have been described, but not spiral. I examined my specimens for long under various powerful lenses, using Reicherts $\frac{1}{15}$, Zeiss $\frac{1}{12}$ and $\frac{1}{18}$ and Powell and Lealands $\frac{1}{12}$ immersion lenses, before determining this point. I have however now no doubt whatever that what at first sight looked like the wavy contractions described by the older observers, are really spiral contractions. This phenomenon I have endeavoured to show in figs. 3 and 4. They were drawn (as were all the figures) with the aid of the camera lucida, and are as exact as I could make them; it is perhaps best shown in Fig. 4, where here and there I think I have succeeded to some extent in

representing the spiral, though not so clearly as could be seen in the specimen itself.

It is to be remarked that the clearest demonstration of spiral contraction is seen in the dorso-ventral fibres, which are attached at both ends, and that in these perhaps the most obvious spiral occurs on either side of the nucleus and close to it. This of course is the thickest portion of the fibre, but spiral contractions are clearly to be seen both in the two branches into which it first divides, and also in the four very fine tendrils into which these ultimately divide.

Thus it may fairly be assumed that this power of contracting spirally is not due to any differentiation of the fibre itself but is an inherent property of the tissue.

The spiral contractions of the circular fibres are less easy to determine because of the difficulty of following each fibre in the bundle, they are much finer than the dorso-ventral fibres, but in certain instances (Fig. 3) the spiral condition is clearly seen. For the same reason I have not found it possible to assure myself that these circular fibres split up into branches and indeed am disposed to doubt that they do so.

The longitudinal fibres I have not been able to examine in a contracted state in my osmic acid preparations, for all the proglottides so preserved have, so far as I have seen, been stretched longitudinally; while in the corrosive and acetic specimens these bundles of fibres are not clearly shown. It is to be noted however that I have seen no nucleus in connection with them.

Thus the main interest of this communication lies in the structure and behaviour of the circular and dorso-ventral fibres; at the same time the similarity in appearance and in consistency of the dorso-ventral and longitudinal fibres in the osmic specimens, and the similar effect which acetic acid has on them both, makes it extremely probable that all the contractile fibres in the body are of the same kind.

In general all the contractile fibres of the cestoda are described as muscles, as for instance by Schmidt (1888, p. 155), though Moniez (1881) referred to them as differentiated fibres which form the so-called muscles of these animals. Pintner (1881, p. 163) specially described the dorso-ventral fibres, and calls them smooth muscles contracting in wavy lines, with a nucleus in the sheath in a non-contractile portion of the muscle.

Hamann, in a paper on *T. lineata* (1881, p. 718) described for that animal;—an outer layer of longitudinal muscles arranged in bundles

beneath the cuticle, which he calls sub-cuticular muscles; an inner layer of longitudinal muscles, in bundles, lying outside a circular layer of muscles; and dorso-ventral muscles. He states that the circular and dorso-ventral muscles are connected at one point with the original cell from which they were formed, the fibre lying on the outside thereof. No cross or longitudinal striae were to be seen in the fibre of the dorso-ventral muscles which he claims branch at their ends and are inserted in the cuticle. He states such muscles are not known in any other *Taenia* except *Bothriocephalus*. He further declares that no trace of the original cell is to be seen in the longitudinal muscles.

Thus the description I have given for *T. elliptica* is in the main similar to Hamann's account of *T. lineata*, the main points of difference being that he does not describe spiral contractions, concludes the dorso-ventral fibres are attached to the cuticle, and is apparently content to consider all the contractile fibres as muscles. It would at any rate appear probable these species are of very similar structure.

The occurrence of spirally contractile fibres has been described in various animals and various organs. The stalk of Vorticella is of course the common example. Hertwig (1880) describes certain fixing or adhesive cells in the ectoderm of the Ctenophora, the base of which is prolonged into a spirally coiled thread. The threads also of the nematocysts of the Coelenterata are also coiled spirally in the cell. Spiral filaments have been described in Spermatozoa by various authors, amongst others by Ballowitz (1891, p. 217); while recently Nicholls (1909, p. 217) figures a cut Reissner's fibre in the spinal cord of the lamprey, which has contracted spirally in a very marked manner.

In none of these cases is the contractile fibre a muscle, while in all of them it may more reasonably be supposed to be some form of elastic tissue, and therefore, as Dendy (1909, p. 217) points out, of the nature of connective tissue.

Schäfer (1891, p. 241) discusses the origin of white and elastic fibres from connective tissue. I cannot claim I have evidence to show whether these contractile fibres in *T. elliptica* are formed by "direct conversion of the protoplasm" of the connective tissue cells, or "by a deposit in the intercellular substance," but if Fig. 2 gives any indication of the origin of these fibres it is certainly in favour of the former view, more especially when the relation of the fibre to the nucleus and its surrounding protoplasm is taken into consideration.

Thus there seems to be little doubt the so-called dorso-ventral and circular muscular fibres of *T. elliptica* are in reality white fibres, not

muscular, and that they are probably derived directly from the connective tissue parenchyma of the animal.

I cannot be certain, for reasons already stated, regarding the longitudinal fibres. Their concentration into bundles indicates they may be more highly specialised than the more scattered contractile fibres, but in the absence of all muscular structure there is presumptive evidence of their similarity with the dorso-ventral fibres. This view is to some extent borne out by the facts that the longitudinal bundles are very variable in size and extent (compare Figs. 3 and 4), that the fibres are very similar to the dorso-ventral fibres at their thickest part, and that the bundles are not enclosed in a sheath.

We have then five stages of the development of contractile fibres before us here. First, the processes of the connective tissue parenchyma cells are contractile; secondly, the superficial and internal processes of the superficial layer of connective tissue cells have increased contractile power; while thirdly, the circular fibres; fourthly, the dorso-ventral fibres; and fifthly, the longitudinal fibres exhibit gradually increasing and more highly specialised development of this function.

On the whole then it may be contended that the supporting structure of the body of *T. elliptica* is to be regarded as a plasmodium of connective tissue, the elements of which are somewhat concentrated on the surface of the body, and are also here and there differentiated into processes or fibres with specially powerful contractile properties; the whole is bounded by a delicate basement membrane and enveloped in a cuticle.

DESCRIPTION OF PLATE XVII.

c. Cuticle.
b.m. Basement membrane.
par. Connective tissue parenchyma.
s.c.t. Superficial layer of connective tissue.
s.l.f. Superficial longitudinal contractile fibres.
l.f. Longitudinal contractile fibres, in bundles, situated deeper in the body.
c.f. Circular contractile fibres.
d.v.f. Dorso-ventral contractile fibres.

Fig. 1. Section through proglottis of *T. elliptica*, showing division of body into dorsal, central, and ventral sections and the situation of the contractile apparatus. (Osmic acid.)

Fig. 2. The specimen from which this section was made was swollen dorso-ventrally and contracted in length. The superficial layer of connective tissue cells are elongated and crowded together, they are connected superficially with the basement membrane by

branched processes, and with the parenchyma of the body and the dorso-ventral fibres by similar branched processes at their inner end (*s.c.t.*). The dorso-ventral fibres are stretched straight, as are also the circular fibres, and are not distinguishable in appearance from the branched processes of the connective tissue parenchyma cells. The nuclear protoplasm of these fibres is apparently but slightly if at all differentiated from the fibre itself. The bundles of superficial longitudinal fibres (*s.l.f.*) and those which lie deeper in the body (*l.f.*) are swollen into masses in which no trace of a fibre is to be seen.

The cuticle is seen separated from the basement membrane. (Corrosive and acetic,—Zeiss $\frac{1}{18}$ imm.)

Fig. 3. The specimen was contracted dorso-ventrally and shows the dorso-ventral and circular fibres contracted into spirals. In this specimen these fibres are seen to be quite distinct from their nuclear protoplasm and of different, much more wiry, consistency than the delicate processes of the connective tissue cells. The dorso-ventral fibres are seen split up into two branches and then into four tendrils, and every portion of the fibre is seen to be capable of spiral contraction.

Both groups of longitudinal fibres are sharply defined. In one place the superficial longitudinal fibres are seen to occupy a space between two of the cells of the outer layer of connective tissue, and in another place a bundle of the deeper lying longitudinal fibres is out of line with the rest: these irregularities I judge to be due to their displacement owing to the dorso-ventral contraction of the proglottis.

The basement membrane is well shown and the superficial processes of the outer layer of connective tissue cells are seen in contact therewith. Owing to the longitudinal extension and dorso-ventral contraction of this proglottis, these latter cells are also contracted and much shorter than in fig. 2, and they are separated one from the other laterally. (Osmic acid,—Reichert $\frac{1}{15}$ imm.)

Fig. 4. The spiral contraction of a dorso-ventral fibre is well shown throughout its whole length (*d.v.f.*), it is seen to pass through a bundle of longitudinal fibres. The differentiation of the fibre from the nuclear protoplasm of its cell is also clearly shown. (Osmic acid,—Reicherts $\frac{1}{15}$ imm.)

Fig. 5. Showing attachment of superficial layer of connective tissue cells to basement membrane, and small bundles of superficial longitudinal contractile fibres within the spaces formed by the superficial branches of these cells. The cells are widely apart and are contracted (compare fig. 2). (Osmic acid,—Powell and Lealand $\frac{1}{12}$ imm.)

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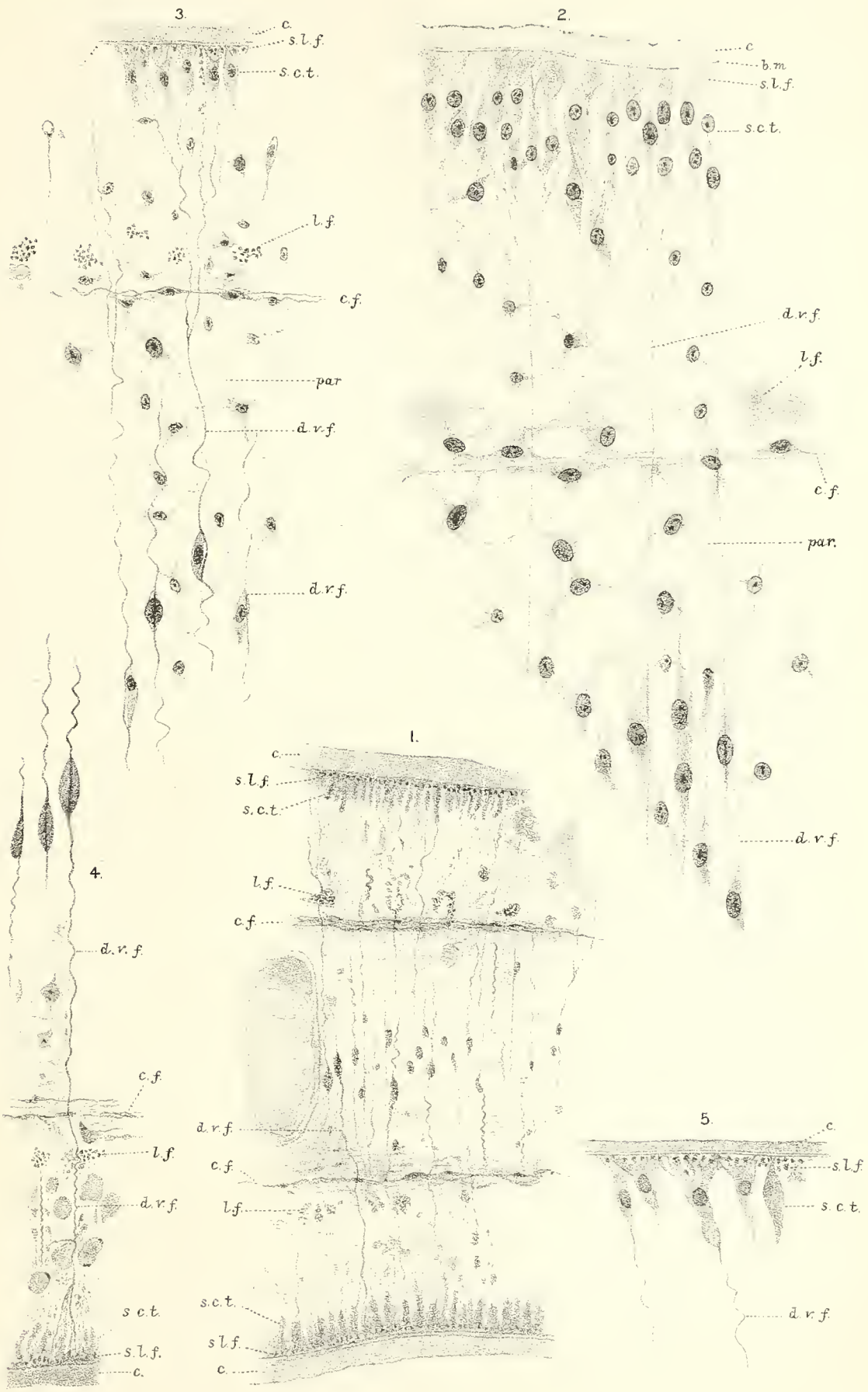

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FIXATION AND STAINING OF *TRYPANOSOMA LEWISI.*

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7 Text Figures.

Since the publication of Moore and Breinl's paper (1908) a new method of wet fixation has been introduced into the technique of protozoological research. It is generally stated that the old method of drying the blood films and fixing them afterwards in absolute alcohol, destroys the minute details of nuclear and protoplasmatic structure; that the preparations made in this way are wholly misleading and the structures do not correspond to those of the living Trypanosomes. This becomes evident when we compare the figures of Moore and Breinl and of Rosenbusch (1909), who studied *Tr. lewisi* with the aid of wet fixation in Flemming's liquid, with those of other authors (Prowazek (1905), Wenyon (1908) etc.) who studied the same subject. The structure of the nucleus is very dissimilar with the two methods. The big karyosome observed by the first authors cannot be found with dry fixation; then only one or more minute granules are to be seen.

If this old method of investigating Trypanosomes and other hematozoa is really not to be trusted, many of the recent papers on the cytology of Trypanosomes (*e.g.* Prowazek's work) are valueless whenever minute cytological details are considered. Before reaching this conclusion it will be useful, however, to compare the different methods of wet and dry fixation and to make sure if this old method is really so bad as many authors assert it to be.

Comparative observations in this respect have been carried out by Minchin (1909) who studied the effect of some fixatives on wet and dried blood films containing *Tr. lewisi*, which were not in the multiplication period since they presented a uniform appearance. When fixing a blood-drop on a cover-slip in osmic acid vapour and staining it with a little methyl-green (so called standard preparations) he found nuclei with minute karyosomes (much smaller than those figured by Moore, Breinl and Rosenbusch), an oval shaped blepharoplast (kinetonucleus) and a flagellum apparently in communication with the latter by its basal end; there is no space between the flagellar base and the blepharoplast as is figured in Moore and Breinl's paper. The standard preparations were used as a control for the following investigation. The Trypanosomes of the preparations which had never been dried were always a little smaller than those of the standard preparations and of the dried films. This shows that drying has not such a marked injurious effect on the cells as is generally believed. The best fixatives are corrosive alcohol (Schaudinn) and osmic acid. Flemming's and Hermann's fluids produce shrinkage of the nucleus (which becomes surrounded by a bright halo) and deformation of the whole cell. The flagellum becomes contracted and a broad space may be seen between its base and the blepharoplast.

Giemsa's method of staining is particularly criticised. It is useful for demonstration but for the investigation of cytological details it is wholly misleading. Much better is iron-hematoxylin. Giemsa's stain produces precipitates which accumulate on the chromatic granules thus increasing their size and forming a diffuse mass, in which no structure and no karyosome is to be detected.

When considering Minchin's results we note that it is not so much the dry fixation as Giemsa's stain which gives misleading preparations and that statements concerning shrinkage and nuclear deformation by dry fixation, such as are contained in many modern textbooks (*e.g.* Doflein 1909, Braun and Lühe 1909) lack confirmation.

Everyone who has studied the cytology of Trypanosomes will regret the loss of the method of dry fixation and especially of Giemsa's staining. The latter stained many a minute structure, without alcohol differentiation being necessary (when only care was taken to check the staining at the right moment). The method was consequently a progressive one, an advantage that the degressive Heidenhain stain does not possess. M. Heidenhain (1907) in his book warns against this method in the following words: "Die Eisenhämatoxylinfärbungen der gewöhnlichen

Art führen meist zu Verklumpungsfiguren der basichromatischen Granula und liefern dabei homogene Färbung der grösseren Teile der Gerüstwerke und der Chromosomen." In view of this statement it is not astonishing when the karyosome in the nucleus of *Tr. lewisi* is so intensely stained with iron-hematoxylin, whereas the surrounding parts of the nucleus are stained a pale-grey colour without any structure.

While studying the literature on *Tr. lewisi* it was necessary to know exactly the value of the methods of dry fixation and Giemsa's staining, to be able to judge some of the recent papers on this subject. This knowledge was absolutely necessary, especially because Minchin says of Prowazek's work: "Moreover Prowazek seems to have based all this theoretical superstructure upon Romanowsky-stained preparations, which in my opinion are altogether false and misleading for minute nuclear structure."

In this note I will not try to interpret any nuclear or protoplasmatic structure observed. They are only considered as an indicator of the value of the different methods of fixing and staining.

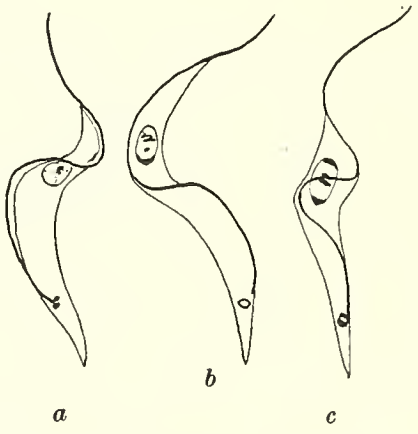

Fig. 1. Standard preparations. Fixation in osmic acid vapour. Unstained.

Standard preparations after Minchin's method were made but not with the same favourable result. I often observed a notable deformation during the fixation in osmic acid vapour. To exclude as perfectly as possible all causes of error, the standard preparations were not coloured, as was done by Minchin. Except for the general form my observations correspond with Minchin's (Text Fig. 1). The blepharoplast is round or oval shaped. Sometimes I was able to detect within it a peculiar structure. It seemed then to consist of two highly refractive granules, united by a less refractive substance (Fig. 1*a* and 1*c*). Generally it was

difficult to decide if the base of the flagellum does or does not reach the blepharoplast, sometimes this was evidently the case, in other specimens the flagellum near the blepharoplast became suddenly indistinct (Fig. 1*b*). The base of the flagellum was often distinctly thickened, so that it seemed to end in a granule (Minchin's blepharoplast).

The nucleus is round or oval, from one to three little granules are to be seen, but no large karyosome as figured by Rosenbusch and Moore and Breinl (Fig. 1*a*, 1*c*). In one case delicate fibrillae were to be seen uniting the central karyosome with the periphery of the nucleus (Fig. 1*a*).

Because of the deformation of the cells in the standard preparations during the fixation, their size does not wholly correspond with Minchin's observations (vide Table II).

The following technique was used to make "wet-fixed never-dried" blood films from *Mus rattus* infected with *Tr. lewisi*, the latter not being in the multiplication period:

The drop of blood taken from the rat's tail was placed on a cover-slip and immediately covered by another cover-slip so that the blood spread between them in a thin layer. Then the two cover-slips were quickly drawn apart and immediately placed (blood film down) on the surface of the fixation-liquid and immersed in it after some minutes. The manipulation of separating the two cover-slips must be performed very quickly to prevent the drying of the thin films.

The fixation-liquids used were the following: Osmic acid in vapour and in solution (2 %) followed by hardening for 24 hours in absolute alcohol; weak Flemming's solution; Hermann's solution; Bouin, Rabl, Rath, Schaudinn and Merkel's fluids; absolute alcohol. To compare these methods with the dry fixation method, blood films were dried and then fixed in absolute alcohol.

Dried films as a rule were stained by Giemsa (Grübler's solution 1 cm., dist. water 10 cm.). As a control dried preparations were also stained with iron hematoxylin. Preparations fixed wet after Schaudinn, Bouin, Flemming etc. were also stained with Giemsa's stain. With dry fixation the staining method was purely progressive, with never-dried preparations it was necessary (to mount in cedar oil) to pass them through alcohol or aceton-xylol mixtures, consequently differentiation was inevitable. To study minute nuclear structures, one must take care to stain only for a short time ($\frac{1}{4}$—1 hour); to procure preparations showing the flagellum well the staining should last longer (1—6 hours). It is desirable to ascertain under the microscope from time to time

how the staining is progressing. No general rules can be laid down because the Giemsa-solutions are often very irregular with regard to their power of staining. With these precautions I never observed the intensely stained structureless nuclei described by Minchin. It is evident that only with dried preparations one is able accurately to determine the required intensity of staining; the process of dehydration always spoils the preparations more or less.

With iron-hematoxylin I obtained the best results after wet fixation. The iron-alum and hematoxylin solutions of Grübler were used. A saturated solution of lithium carbonate was added to the hematoxylin till a claret colour was produced (2—3 drops suffice). Preparations were left in the two solutions for 24—48 hours. The differentiation in iron-alum was controlled under the microscope; it took 1—3 minutes.

Other stains, for instance hemalum (P. Mayer) and Ehrlich-Biondi's stain (after Merkel's liquid), gave rather indifferent results. Delafield's and Carmin stains I found wholly useless.

I will now discuss the influence of the different fixative and staining media on the structure of the nucleus, the blepharoplast, the flagellum (with the basal granule), the cytoplasma and the general cell form.

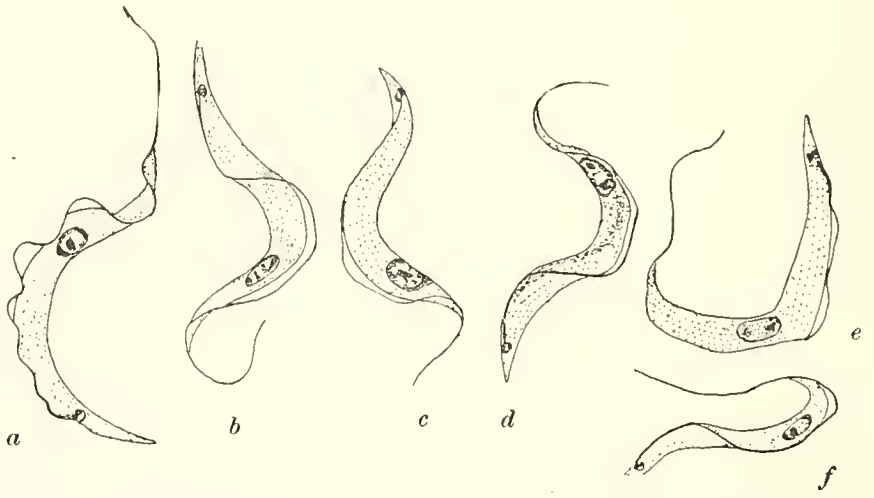

Fig. 2. Dried blood-films alcohol-fixation. *a—e*, Giemsa's stain, *f*, Heidenhain's stain.

1. *Nucleus.* In dried films, fixed with absolute alcohol and stained with Giemsa ($\frac{1}{2}$—1 hour) the nucleus is oval shaped. It is composed of a pink coloured matrix, in which a dark red substance is embedded. The first is structureless or has a more or less distinct alveolar structure (Fig. 2*a*—2*d*). The dark red substance consists of a large central granule and two smaller ones situated in the periphery, having the

appearance of a nuclear membrane (Fig. 2*a*, 2*c*); or two granules of the same size are present, each of them may be divided (Fig. 2*d*, 2*e*). No real nuclear membrane is to be seen. The peripheral chromatin cannot be considered as such, often it is only found at the two poles of the nucleus. The preparations fixed by this method were also stained with iron-hematoxylin. No structure of the achromatin of the nucleus was to be seen, the chromatic granules were embedded in a structureless, pale-grey matrix. As in Giemsa stained preparations one or more central granules and a more or less complete peripheral mantle of chromatin was to be seen (Fig. 2*f*).

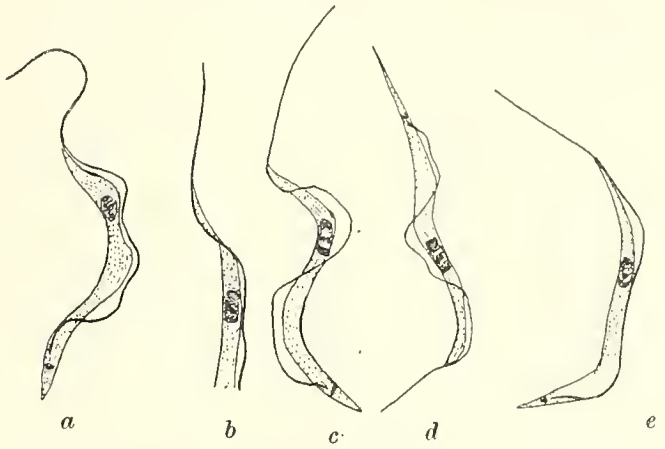

Fig. 3. Fixation in Schaudinn's corrosive alcohol. Heidenhain's stain.

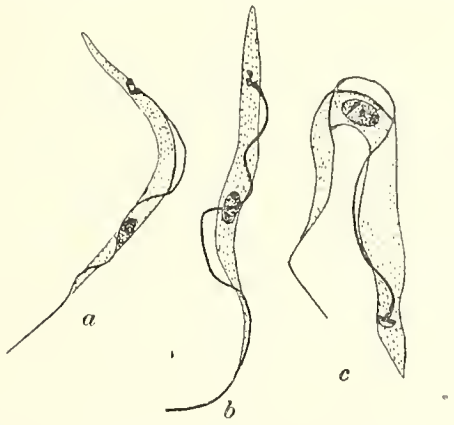

Fig. 4. *a* and *b*, as Fig. 3, *c* as Fig. 2.

Fixation with Schaudinn's fluid (sublimate and absolute alcohol) followed by Heidenhain's stain gave the same results as alcohol fixation of dried films stained in the same way. The achromatin is without any structure. A central agglomeration of chromatin is present; at the

periphery of the nucleus two chromatic rodlets may be seen, having the appearance of a membrane (Fig. 3*d*, 3*e*, 4*a*). Often only two chromatic granules (Fig. 3*e*, 3*b*, 4*b*), or three of different size (Fig. 3*a*) are present. When comparing Fig. 2*a*, 2*b*, 2*d* with Fig. 3*e*, 4*a*, 4*b*, the similarity of the results obtained with dry fixation-Giemsa's stain and with sublimate fixation-Heidenhain's stain, will be evident.

Giemsa's stain following fixation in Schaudinn's liquid does not give satisfactory results. Good preparations for demonstration may be obtained, but for the study of the nuclear structure they are useless. No better results follow staining by Giemsa's recently described method (1909). The nucleus looks like a dark red spot, in which no structure whatever is to be seen.

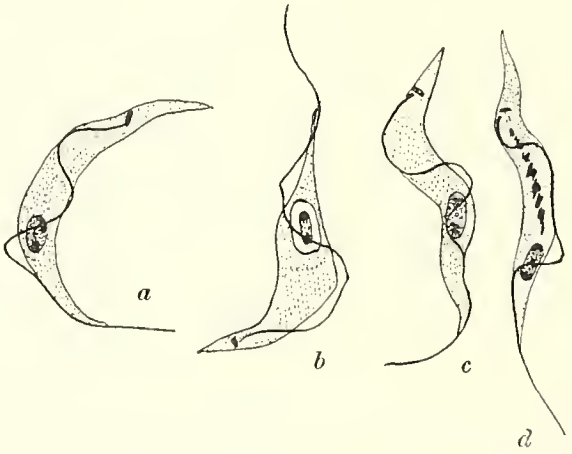

Fig. 5. *a*, *c*, Hermann's fixation; *b*, *d*, Flemming's fixation, followed by Heidenhain's stain.

Fixation with Flemming's and Hermann's solutions produces more or less considerable shrinkage. The volume of the nucleus is reduced and the latter is surrounded by a bright halo, thus simulating a nucleus with a large karyosome as may be found among Amoebae. Hermann's solution gives better results than Flemming's. An advantage of these fixatives is the beautiful Heidenhain stain, which may afterwards be obtained. The nucleus is stained very deeply and so a very sharp differentiation is possible. The nuclear structure is the same as that obtained by the two methods described, as may be seen when comparing Fig. 5*a*, 5*c* (Hermann's fixation) and Fig. 5*b*, 5*d* (Flemming's fixation); Giemsa's stain gives no better results than after corrosive-alcohol. Its only advantage is that it gives sharp outlines to the nucleus.

Very sharp differentiation may be obtained with Heidenhain's stain after fixation in Rath's solution; no shrinkage of the nucleus was

observed. This method of wet fixation is highly recommendable, because it possesses the advantages of Flemming's and Hermann's solutions without their disadvantages. Fig. 6*a*, 6*d* show that the nuclear structure after Rath's fixation is the same as that already described. Fig. 6*a* presents a very distinct division of the two chromatic granules. Giemsa's

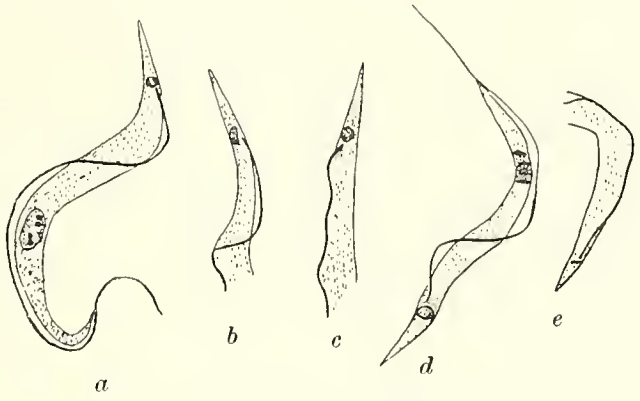

Fig. 6. Rath's fixation. Heidenhain's stain.

stain after Rath's fixation does not give such bad results as are obtained by other methods of wet fixation, but they are inferior to those obtained with dried films. Rabl's and Bouin's liquids are not so useful as Rath's but give rather good results. Delafield's hematoxylin and Mayer's hemalum gives mediocre staining after Bouin fixation. The nuclear structure brought out by these methods is similar to that already described.

Merkel's solution gives very inferior preparations. Not only a considerable shrinkage is to be observed, but no sufficient staining with iron-hematoxylin can be obtained. Only Ehrlich-Biondi's mixture gives rather distinct nuclear figures, but the shrinkage is so considerable that the preparations cannot be trusted.

Wet alcohol fixation with Giemsa's or Heidenhain's stains gives similar results to those obtained by other methods (Fig. 7).

I could not obtain good preparations either with iron-hematoxylin, or with Giemsa's stain after fixation in osmic acid (vapour or 2 % solution) followed by hardening in absolute alcohol. My preparations showed only the general outlines, rarely the internal structure of the nucleus was to be seen.

When comparing the results obtained by the different methods of fixing and staining I am unable to confirm Minchin's statement that Giemsa's stain is useless for the study of minute nuclear structure. On

the contrary, after dry fixation, it gives figures so sharp as may rarely be obtained with iron-hematoxylin. Moreover the achromatin is better differentiated and the method being a progressive one is more reliable than the degressive method of Heidenhain. As for the fixation after drying I never observed any sign of shinkage, of destruction, or even of the slightest dislocation of the nuclear structure. The figures observed in preparations made after this method are perfectly similar to those produced by the best methods of wet fixation.

To be able to judge of the influence of the fixing fluids used on the shape of the nucleus I tried to obtain a numerical expression for it. Nuclei of Trypanosomes fixed by the different methods and stained by Giemsa (this stain marking very distinctly the outlines of the nucleus) were drawn with the camera lucida. On the same piece of paper used to draw the Trypanosomes a scale of 10 μ was drawn (using an object-micrometer) so that the nuclei might be measured after they had been subjected to different methods of fixation. The following table gives the results of those measurements.

This table confirms the results of the study of nuclear structure. Dry fixation, Corrosive alcohol and Osmic acid give the best results. Absolute alcohol and the Picro-corrosive mixtures are a little inferior, Hermann's and Flemming's liquids are not favourable.

TABLE I.

Showing the average size of the nucleus in Tr. lewisi after using different methods of fixation. Films stained by Giemsa.

Fixative	Longitudinal diameter	Transverse diameter
Corrosive-alcohol	2·1	0·9
Dry fixation	2·3	0·9
2 % osmic acid	2·0	0·8
Absolute alcohol	1·9	0·7
Rath's liquid	1·8	0·7
Rabl's liquid	1·8	0·7
Hermann's liquid	1·8	0·4
Flemming's liquid	1·8	0·5

2. *Blepharoplast.* (Minchin's kinetonucleus.) The structure of the blepharoplast after fixation and staining by different methods may vary considerably in appearance depending upon the total or partial staining of its component parts.

After fixation with corrosive alcohol and staining with iron-hematoxylin, the blepharoplast presents itself as a rodlet (Fig. 3)

which may often be found to consist of two granules (Fig. 4*b*). A closer examination shows that the two granules are embedded in an achromatic globule, coloured pale grey, but generally left uncoloured after differentiation in iron-alum, so that the blepharoplast seems to consist only of the chromatic rodlet or the diplosome. Perhaps it will be useful to point out again that this diplosome cannot be considered as a sign of division, the Trypanosomes being no more in a division period.

Similar but more distinct results are to be obtained after Rath's fixation and iron-hematoxylin. In some cases the blepharoplast has the shape of a rodlet as in Fig. 6*e*; Fig. 6*c* shows, however, that only part of the blepharoplast had been stained in the foregoing figure. In Fig. 6*c* the blepharoplast is composed of an intensely staining rodlet partly surrounded by the achromatic substance. Fig. 6*a*, 6*b* present the same structure but the rodlet appears here as a diplosome. In Fig. 6*d* only a grey coloured globule is to be seen, not showing any internal structure, the colour has in this case been completely removed.

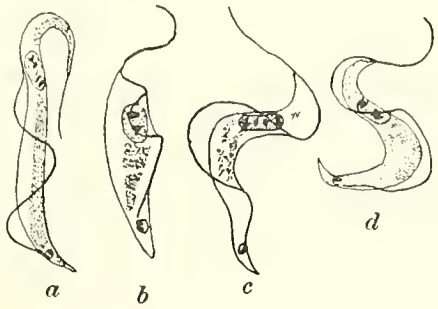

Fig. 7. Alcohol fixation of never-dried preparations. Giemsa's stain.

Flemming's and Hermann's liquids are useless for the study of the blepharoplast, Giemsa's stain after alcohol-fixation of dried films, wet alcohol fixation or fixation in osmic acid is very useful to show the minute details of the structure of the blepharoplast (Fig. 2*a*—2*d*, Fig. 4*c*, Fig. 7) because the chromatic and achromatic components are stained in different varieties of red. Care must be taken that the staining does not last too long, if this precaution is not observed chromatic and achromatic substances are stained with equal intensity and the blepharoplast looks in such cases like a large structureless globule, as has been figured by Minchin. Giemsa-stained preparations produce the same structures of the blepharoplast as those stained by Heidenhain's method as may be seen when comparing the text figures. Consequently it is evident that dried films fixed with absolute alcohol and stained by Giemsa present the same minute structure of the blepharoplast as do the different methods of wet fixation and staining.

The structure of the blepharoplast described here was already known from Prowazek's and Wenyon's (1908) work. Minchin asserts that the blepharoplast is structureless.

3. *The flagellum and its basal granule.* Minchin states that the base of the flagellum does not reach the blepharoplast, when films are dried before fixation and that consequently a more or less extensive cleft may be observed between the two. In my preparations (dry fixation, Giemsa's stain) the following peculiarities could be observed. Often the flagellum seemed to be joined directly to the blepharoplast (Fig. 2*b*—2*d*); in other cases the basal part of the flagellum was stained with less intensity than the other parts of the flagellum, so a chromatic and an achromatic part of the flagellum could be distinguished, the first being united to the blepharoplast by means of the latter (Fig. 2*a*, 2*e*). Sometimes, but by no means as a rule, the base of the chromatic flagellum seemed to be broadened there where the achromatic filament commences. I think that this broadened end must be considered as the basal granule (Minchin's blepharoplast).

Iron-hematoxylin after fixation with corrosive alcohol gave similar figures but the achromatic filament uniting the basal granule and the blepharoplast (Fig. 4*a*, 4*b*) was but rarely observed. Generally the basal granule was superposed on the blepharoplast (Fig. 4*a*, 4*b*), sometimes a free space was left between the two (Fig. 3*a*). The basal granule was clearly to be seen with Heidenhain's stain even better than with Giemsa's. The increase in size of the basal granule after Giemsa's stain, as pointed out by Minchin, could never be observed.

4. *The Cytoplasma.* After fixation by Flemming's fluid a distinct alveolar structure of the cytoplasma may generally be observed. With other methods (dry fixation, corrosive-alcohol, Rath) no such structure can be seen, so I presume that it is an artefact.

After wet alcohol-fixation the cytoplasma assumed a deep blue colour and did not completely fill the space enclosed by the periplast, leaving a large part of it uncoloured. The coloured and uncoloured parts were sharply separated (Fig. 7*a*). I think this structure may be identified with Minchin's "Periplast line." The relation of the coloured and uncoloured part is not always such as I have here described. Sometimes the coloured part is confined to a narrow median space (Fig. 7*b*) and may be composed of two parallel longitudinal filaments (Fig. 7*c*). Here the "contraction" of the protoplasma is very intense, much more so than after Flemming's fixation although considerable alteration of the cell form occurs in the latter case. It is therefore by no means clear why after such intense plasmatic contraction the outlines of the cell are so

little altered. Fig. 7*d* may furnish the explanation. Here the darkly coloured plasma is also to be seen, but its outlines are not so sharp as in Fig. 7*a*. The remaining parts of the cell are not empty as they seemed to be in the foregoing figures, but filled with plasma of a pale-blue hue. In Fig. 7*a*, 7*c* this part of the cytoplasma has not been stained. I think it probable that this structure indicates a morphological differentiation in the cytoplasma. It corresponds with some observations made with dried, Giemsa-stained preparations, where a darkly staining plasmatic band between nucleus and blepharoplast may frequently be seen (Fig. 2*d*).

These structures were equally distinct with Heidenhain's stain, but only after wet fixation with alcohol. After Flemming's fixation a median darkly staining band composed of irregular granules between nucleus and blepharoplast was to be seen (Fig. 5*d*). Perhaps this string of granules has something to do with the periplast line. I think they are to be identified with Minchin's "chromatoid granules" but they did not stain well with Ehrlich's or Delafield's hematoxylin, so I am not sure of it.

When comparing the influence of the different methods of fixation and staining on the cytoplasma we may conclude that the dry fixation Giemsa method is not inferior to the others.

5. *General cell form.* To be able to express the value of the different methods of fixation in numbers, I measured a quantity of Trypanosomes fixed with different fluids, 10 cells for each method. Like Minchin I measured:

(*a*) The part of the cell posterior to the posterior end of the blepharoplast (post-nuclear).

(*b*) The part between the anterior pole of the nucleus and the posterior end of the blepharoplast (intranuclear).

(*c*) The part between the anterior pole of the nucleus and the end of the undulating membrane (prenuclear).

(*d*) The greatest breadth.

(*e*) The length of the whole cell (free flagellum excepted).

Table II gives the results of these measurements.

The results of these measurements correspond exactly with those of the morphological study and of the measurements of Table I. Flemming's and Hermann's stains produce general deformation, so does wet alcohol fixation, but this is not so marked. The best preparations are to be obtained with alcohol fixation of dried films or with fixation in corrosive-alcohol, osmic acid and Rath's fluid.

TABLE II.

Dimensions	Standard preparation, own observation	Standard preparation, after Minchin	Corrosive alcohol	Osmic acid 2%	Alcohol, dried films	Alcohol, wet films	Rath's liquid	Hermann's liquid	Flemming's liquid
Post-nuclear	2·6	4·5	2·4	2·6	2·5	1·8	2·2	2·6	2·9
Intranuclear	8·5	12·22	8·4	9·8	10·7	8·1	10·6	8·7	7·9
Prenuclear	4·0	7·04	4·7	5·6	6·8	3·2	5·5	4·7	3·8
Greatest breadth	1·6	1·64	1·1	1·6	1·7	1·5	1·5	1·8	2·2
Whole length	15·1	23·8	15·5	18·0	20·0	13·1	18·3	16·0	14·6

Concluding remarks. The opinion generally accepted at present, that the old method of fixing and staining is misleading when used for the study of minute cytological details, is incorrect with regard to *Trypanosoma lewisi.* This method is at least equivalent to the best methods of wet fixation and gives, followed by Giemsa's stain, a differentiation of the structure of nucleus and blepharoplast which is decidedly superior to Heidenhain's stain, if only overstaining is prevented. Consequently the papers referring to *Tr. lewisi,* issued before 1907 and based on the old fixing and staining methods, are fairly reliable. Of course this statement holds good only for *Tr. lewisi*; with other haemoflagellates, especially the larger ones, things may be different as may be concluded from M. Robertson's description (1909).

It will be advisable when studying hematozoa to use first of all the method of dry fixation with subsequent staining by Giemsa's method. Of course it is always advisable not to be content with one method but to test the results of it by others; but to reject the old method as untrustworthy is wholly unjustifiable and would mean a great loss for microscopical technique.

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THE LEUCOCYTOZOA,

A REJOINDER TO Mr C. M. WENYON.

By ANNIE PORTER, B.Sc.

In the last number of *Parasitology* (Vol. III. No. 1) Mr Wenyon has taken exception to an article of mine on the *Leucocytozoa*, published in *Science Progress*, October, 1909. It has been a matter of wonder to many protozoologists as well as to myself, why Mr Wenyon did not reply to my article in *Science Progress* itself, and thereby appeal to the readers whom he accuses me of having misled, for he states (*Parasitology*, p. 65) that my "survey of the group is inaccurate and contradictory."

The character of my article in *Science Progress* is well summed up in my concluding paragraph on p. 265. "Such is a general survey of a very interesting group, the *Leucocytozoa*, which, perhaps, is not homogeneous, as the forms in birds differ in some respects from those occurring in mammals. This survey is the first that has been attempted, and it is hoped that it may draw attention to and stimulate research upon an interesting group of parasites which are probably more numerous than we know at present. They should be searched for among reptiles, amphibia and fishes." This paragraph, along with the major part of my article, Mr Wenyon has chosen largely to ignore.

Mr Wenyon's chief accusation is that I have confused the avian *Leucocytozoa* with the haemogregariniform parasites found in the leucocytes of mammals. This is grossly inaccurate, for on p. 264 of my article, I state that "as the structure and life-history of avian *Leucocytozoa* are still subjects of controversy, and as the name *Leucocytozoon* was first applied to the parasites of birds, and Lühe seems to restrict the name thereto, the generic name *Leucocytogregarina* might be used for the highly specialised parasites of mammalian leucocytes, which have a different habitat from the strict Haemogregarines of red corpuscles." I think from the foregoing quotation, it will be quite obvious to my readers that I have not confused the avian and mammalian parasites, for both of which the name *Leucocytozoa* has been used by many authors, and it was necessary, in a somewhat popular article, to deal with all the parasites that had at any time been termed *Leucocytozoa*.

Doflein (1909) adopts a similar view in his recent treatise on the Protozoa.

At the end of his article, after accusing me of many mistakes that I have not committed, Mr Wenyon is forced to admit that "the suggestion at the end of the review of a new generic name seems to indicate some doubt even in Miss Porter's mind as to the validity of this grouping." Needless to say, had I not been fully cognisant of differences between the avian and mammalian parasites, I should not have put forward the new descriptive name *Leucocytogregarina* for the haemogregariniform parasites of the leucocytes of mammals.

In several places in my article (*e.g.* pp. 256, 264) I state that the structure and life history of avian *Leucocytozoa* are still subjects of controversy; therefore they are open to differences of opinion. Mr Wenyon ignores this, and proceeds to use such loose statements as that on p. 64 where we read that "It is evident that Miss Porter has no knowledge of the *Leucocytozoon* of birds for her survey of the group is inaccurate and contradictory." Such dogmatic assertions regarding a worker with whom he is unacquainted personally, and of the extent of whose information he has no first-hand knowledge, need no further comment from me, except that scientific research is considered to engender a spirit of toleration accompanied by a broad outlook, hardly compatible with such assertions as the one quoted above. It may, however, give some slight satisfaction to Mr Wenyon, as well as confidence to scientific workers generally, to know that I have an intimate personal knowledge of the *Leucocytozoa* occurring in the domestic fowl, the sparrow, the lark, the Scotch grouse and the guinea fowl—some of which parasites have never been described or recorded in England till now. I fear, therefore, that in this case it is the statement of Mr Wenyon that is "inaccurate"—as well as unwarranted.

I must crave the indulgence of my readers in that I have no choice but to enter more fully into certain of the criticisms passed on the short general review that I wrote last year.

An appreciable proportion of Mr Wenyon's paper consists in the setting forth of what he is pleased to term the *characters* of *Leucocytozoon* and of the leucocytic parasites of mammals.

Regarding the third of these characters (this *Journal*, Vol. III. p. 65) Mr Wenyon is wrong in laying great stress on the *Leucocytozoa* of birds causing the host cell to assume a characteristic spindle form. Mathis and Léger have described *Leucocytozoon caulleryi* from the domestic fowl of Tonkin, and *L. marchouxi* from *Turtur humilis* neither of which causes any assumption of a spindle shape by the host cell.

Laveran and Lucet state that the host cell of *L. smithi*, parasitic in the turkey, may be either oval or greatly elongated. As stated above, I have seen *Leucocytozoa* living in the common fowl in England, a parasite probably the same as that of Mathis and Léger, and it does not produce spindle elongation of the host cell. The property of producing marked deformation of the host cell, then, breaks down as a character of avian *Leucocytozoa*.

Again the fourth character given is that male, female and young parasites, possibly immature gametocytes, are found in the circulating blood. I would suggest that there is another possibility—that some of these forms are not gametocytes but are schizonts. This possibility has been overlooked.

With regard to the seventh character Mr Wenyon states that the parasites never leave the host cell to move about in the blood plasma. Because Mr Wenyon has not seen this phenomenon, it does not follow that it does not occur. Further, I would ask how the primary infection of the host cell takes place and how the periodicity exhibited by *L. caullery*i described by Mathis and Léger is to be explained, were there not free forms in the blood at some stage.

A still more astonishing character (!) is given when it is stated that the "asexual mode of reproduction is unknown." It is surprising that lack of knowledge of various phases of the life history of any organism should be quoted as evidence regarding the character of that organism. Apart from that, the statement is inaccurate, for schizogony of *Leucocytozoon lovati* of the grouse is now known. (See *Abstracts Proc. Zool. Soc.* (1910) No. 84.)

Had Mr Wenyon taken note of the sections of my article dealing with the general description, movements, comparative morphology, multiplication and reproduction, he would have acknowledged that most of his "characters," and particularly those of the leucocytic parasites of mammals, were clearly set forth therein, but without the dogmatic assertion or suggestion that they were invariable features or characters of the organisms. When Mr Wenyon can refer us all to a generally accepted complete life cycle of an avian *Leucocytozoon* as well as to an equally generally accepted complete life cycle of a mammalian *Leucocytogregarine*, then it will be time to draw up tables of differences between the various parasites under discussion. Meanwhile such character differences as those used by Mr Wenyon are very artificial, and as shown, often quite inaccurate.

A side issue is raised when the question of *Leishmania* and *Herpetomonas* is discussed by my critic. But though irrelevant to *Leucocytozoa*

it is of some service. One reason for calling the haemogregariniform parasites of leucocytes by the generic name *Leucocytozoon* was the high degree of specialisation involved in their habitat. Mr Wenyon, ignoring this specialisation, asks can mere habitat be regarded as a generic character—and himself supplies the answer in the affirmative by quoting the alternation of hosts in the case of "*Leishmania*," "a profound distinction which undoubtedly justifies its inclusion in a distinct genus" (from *Herpetomonas*). I do not wish to confuse the issue regarding *Leucocytozoa* by entering into this controversy. Capt. Patton and Mr Wenyon among others can settle that question. But the high degree of specialisation shown by the haemogregariniform parasites of mammalian leucocytes is surely more than a matter of "mere habitat."

Further, with regard to habitat being a specific character, Mr Wenyon has some slightly personal remarks, which are entirely his own, and from which I most strongly dissociate myself. He says "Miss Porter follows James and Patton, who are stated to have the advantage over Laveran and Mesnil, of first hand knowledge of the group. Apparently Miss Porter imagines that Laveran and Mesnil and possibly others have not this first hand knowledge, but I can assure her that in this she is mistaken." The inference is that of my critic and not mine; nor did I state that James and Patton had advantage over Laveran and Mesnil, but that they had first hand knowledge.

It would not be advisable to continue an argument advanced in the "Remarks on the genus *Leucocytozoon*" regarding the association in classification of the *Leucocytozoa* of birds with such parasites as those of malaria. Capt. Patton considers the avian *Leucocytozoa* to belong to the genus *Haemamoeba*, and I need only say, that in so doing, he follows Laveran in this association,—a scientist previously quoted by Mr Wenyon as an authority on the *Leucocytozoa*.

With regard to the priority of the name *Hepatozoon* of Miller, it should be stated that the first observed and type species of the haemogregariniform parasites of the leucocytes of mammals, was that parasitic in the dog, and named *Leucocytozoon canis* by James, a life cycle of which in the dog-tick has been described by Christophers. *L. canis* being the type species, then *Hepatozoon*, "a monotypic genus" (Miller), and the name of a parasite in the leucocytes of white rats, does not hold as a generic name for these leucocytic parasites of mammals, and even Mr Wenyon writes that "Miller suggests for the rat parasite the name *Hepatozoon perniciosum*." Miller did not suggest that *Hepatozoon* should be the generic name for all the *Leucocytozoa* of mammals, but even stated that as the complete life cycle of many of the parasites

had not been established, by including his parasite in any of the already existing genera, "the danger is present of giving a total misconception to the life cycle of other forms." Again, Miller's work on *Hepatozoon* has not yet been confirmed, and there is the possibility that the phases described as those of *Hepatozoon* in the mite, *Lelaps echidninus*, may be phases in the life history of a parasite natural to the mite itself. This matter I have already discussed sufficiently in *Science Progress*. Until Miller's work on *Hepatozoon* be confirmed, it seems to me well to reserve definite judgment on the sporogony of mammalian *Leucocytogregarina*.

The relevancy of certain remarks in the footnote on p. 66 regarding *Piroplasma* and Trypanosomes, as well as their accuracy, may also be questioned. Two main chromatic bodies occur in a Trypanosome. The larger of these is commonly called the nucleus or trophonucleus, the smaller is generally designated the blepharoplast or kinetonucleus. The latter body is near the origin of the flagellum and is generally considered to be concerned with regulating the function of movement. Mr Wenyon calls this small body the "micronucleus[1]," a term usually associated with the small mass of generative chromatin of a Ciliate such as *Paramoecium*, which body has no connection with locomotion.

Again, the law of priority is not a strict law (proved hypothesis), but a convention or rule, which, in addition to serving its primary purpose, has brought about much confusion and controversy, and must, of necessity, with increase of knowledge, be displaced. For instance, the familiar *Coccidium* is no longer designated as such owing to the strict application of the law of priority, while the equally well known *Piroplasma* should give place to *Babesia*. The vexatious use of the rule of priority has already been protested against by many of the greatest living zoologists, Lankester, Shipley, Minchin and Boulenger among others, and will probably be soon superseded by less rigid yet equally correct conventions. In the meanwhile, it is neither presumption nor crime to suggest a more reasonable nomenclature, for the world has long since dispensed with such inelastic codes as "the law of the Medes and Persians which altereth not."

[1] The reproductive character of a micronucleus has been emphasised in the classification of the Protozoa as set forth by Doflein and by Hickson. The Protozoa are ranged in two great groups :—

I. The *Plasmadromata* (Doflein, 1901), Protozoa in which the nucleus is not separated into reproductive (micronncleus) and non-reproductive (macronucleus) portions.

II. The *Heterokaryota* (Hickson, 1903), *Ciliophora* of Doflein, Protozoa possessing cilia either throughout life or only in the early stage of the life cycle, bnt always with a micronucleus which is entirely reproductive in function; and a macronucleus which is trophic and kinetic.

There are other remarks in Mr Wenyon's paper to which exception might easily be taken. Some of these arise from consideration of sentences in my article isolated from their context. Such a procedure is more compatible with the political arena than with scientific research. I will not weary my readers with further details.

In conclusion, I would cordially thank Mr Wenyon for the great honour he has done me in bringing into prominence my modest article on the *Leucocytozoa*, wherein I naturally considered all the parasites which had ever been so designated. The article, as Mr Wenyon knows quite well, was of a slightly popular nature, in which controversy—that adds glory to neither party—could not be set forth at length. At any rate, my article has served one purpose at which I aimed, that of drawing "attention to an interesting group of parasites."

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